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June 2021
ISSN 1996-0808
DOI: 10.5897/AJMR
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Vol. 15(6), pp. 272-277, June, 2021
DOI: 10.5897/AJMR2020.9404
Article Number: FBDF88066901
ISSN: 1996-0808
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African Journal of Microbiology Research

*Full Length Research Paper*

# Shelf stability of processed cocoyam flour during storage at room temperature (28.0 ± 2°C) for a period of four months

**G. I. Okwu*, A. R. Akpe and A. A. Osawaru**

Department of Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Received 18 August, 2020; Accepted 8 December, 2020

**The shelf life study of dry milled cocoyam flour packaged in low density polyethylene was carried out for a period of 4-month. Microbiological, nutritional, physicochemical quality characteristics and aflatoxin content were evaluated. The total viable bacterial counts ranged from $1.6\times10^3$ - $4.8\times10^5$ cfu/g while the total viable fungal count increased from $5.0 \times 10^1$ - $3.8 \times 10^5$ sfu/g. The bacteria isolated include *Bacillus* species, *Bacillus subtilis, Proteus* species, *Staphylococcus epidermidis, Streptococcus pyogenes, Micrococcus luteus, Klebsiella* species, *Staphylococcus aureus, Pseudomonas* species and *Staphylococcus saprophytic.* Fungal genera isolated include *Penicillum* species, *Aspergillus flavus, Aspergillus niger, Fusarium*, *Mucor* and *Rhizopus* species. Gradual decrease in pH (6.40 ± 0.001 to 4.17± 0.01) and noticeable increase in titrateable acidity (0.024 ± 0.003 to 1.17 ± 0.01%) were observed during storage. There was an increase in moisture content while carbohydrate, protein, fat, crude fibre and ash were found to decrease during storage. Aflatoxin B1 and B2 content from 0 h to the 4th month were (0.020, 0.006) and (0.097, 0.063) µg/kg respectively. The presence of aflatoxin $B_1$ and $B_2$ is of public health concern. There is need for improved processing, handling techniques and good hygiene practices to ensure safety of the finished product.**

**Key words:** Cocoyam, shelf life, room temperature, aflatoxin content, nutritional analysis.

## INTRODUCTION

Cocoyam is an herbaceous perennial plant which belongs to the family Araceae. Cocoyams are originally from the tropical and sub-tropical countries and studies reveal that cocoyam is among the least studied root plants. Some species include *Xanthosoma sagittifolium, Amorphophallus titanum* and *Colocasia esculenta*. This species, *Xanthosoma sagittifolium*, is food for over 400 million people worldwide and is the most consumed in West Africa (Boakye et al., 2018). According to Onyeka (2014), Africa is the major producer with West and Central Africa, notably, Nigeria, Ghana, and Cameroon contributing to over 60% of the total African production. Nigeria is the world's largest producer of cocoyam. The average production figure for Nigeria is 5.400 Metric Tonnes which accounts for about 37% of total world's output of cocoyam (FAO, 2012). It is nutritionally

*Corresponding author. E-mail: grace.okwu@yahoo.com.

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superior to yam and cassava in terms of its digestibility, contents of crude protein and essential minerals, such as Ca, Mg, and P (Chukwu et al., 2012). All parts of the cocoyam (corm, cornel, leave and flower) are edible and the corms contain approximately 25% starch and eaten mainly as thickeners, purees or whole (Ejoh et al., 2013).

It is used in the treatment of diabetes, prevention of cancer and as food for the aged people, individuals and children (Kundu et al., 2012).

The crop has assumed nutritional and industrial significance in flour industries (Onwubuya and Ajani, 2012). Cocoyam flour can be used in the production of bread, cakes and also in chin-chin production.

Cocoyam is a great source of dietary fibre and starch that can generate energy to the body (Adekiya et al., 2014); also an adequate source of potassium (Nnabuk et al., 2012).

Previous works have been carried out on the functional properties of raw and precooked taro (*C. esculenta*) flour (Tagodoe and Nip, 1994), starch structure and some properties of cocoyam (Sefa-Dede and Sackey, 2002), production of ethanol from cocoyam (*C. esculenta*) (Braide et al., 2011) and effects of processing on energy value, nutrient and anti-nutrient components of wild cocoyam (Olajide et al., 2011). Despite the increasing demand and usage of cocayam in Nigeria, there is little scientific information on the quality characteristics, shelf stability and packaging existing in literature, hence the need for this present work.

## MATERIALS AND METHODS

Healthy red coloured cocoyam (*C. esculenta*) tubers (100 kg) were purchased from different vendors at Iruekpen market, Edo State, Nigeria, and placed in two sterile Hessian bags of 50 kg each. Thereafter, it was taken to Ambrose Alli University Microbiology laboratory where it was immediately processed, packaged and analyzed.

### Preparation of sample

The cocoyam tubers were peeled, washed, sliced thinly, and oven dried at a temperature of 65°C for 2 h. Thereafter, it was ground into powdered form with a milling machine and immediately packaged in a low density polyethylene (LDPL) (50 g per pack), sealed, labeled and kept on the shelf at ambient temperature (28 ± 2°C) for further analyses.

### Microbiological analysis

Samples of the packaged cocoyam flour were analyzed microbiologically. At intervals (1 week, 2 weeks, 1 month, 2 months, 3 months and 4 months), 10 g of each sample was homogenized in 90 ml of sterile distilled water for 2 min to obtain stock solution. A further ten-fold serial dilution was done up to $10^{-8}$ for colony counts. Aliquots (1 ml) of appropriate dilutions were aseptically pour-plated in nutrient agar for isolation of bacteria and on acidified potato dextrose agar for isolation of fungi under aseptic conditions. The nutrient agar plates were aerobically incubated at 37°C for 24 to 48 h, while the potato dextrose agar plates were incubated at room temperature (28.0 ± 2°C) for 3 to 5 days. At the end of incubation, the colonies were enumerated and recorded. The bacterial isolates were purified, characterized and identified using series of cultural and biochemical tests as described by Ochei and Kolhatkar (2008). The fungal isolates were identified microscopically using lacto-phenol cotton blue test.

### Physicochemical and nutritional analysis

The moisture content of the samples was determined according to Cole (2002). This is an oven dry method in which weight of the various samples was placed in previously weighed watch glass and the initial weight noted. Thereafter, it was placed in a vacuum oven (Gallenkamp) at 95±2°C. At intervals, the sample was brought out and weighed, until the weight became constant (final weight). The difference between the initial and the final weight was recorded as the moisture content. The pH readings were obtained using a digital Jenway Model 3510 benchtop pH meter. The available carbohydrates, proteins, lipids, ash, crude fibre and titratable acidity (TA) were also determined according to AOAC (2008) methods.

### Aflatoxin determination

The extraction, detection and quantification of aflatoxin were done according to the method of Jonathan and Esho (2010). Known weight (5 g) of sample was added to 7 ml of distilled water and 25 ml of chloroform, the mixture was shaken and left for 30 min after which the solution obtained was filtered using a Whatman No. 1 filter paper. Extract was obtained and evaporated to dryness to a volume of 5 ml on a hot water bath (Gallenhamp, England). 0.5 ml of the reconstituted extract with chloroform was spotted on a pre-coated 20 × 20 $cm^2$ thin layer chromatography (TLC) plate along with aflatoxin standard of known concentration. Developed TLC plate was air dried at ambient temperature (28 ± 2°C) and aflatoxins were detected under UV light at wavelength of 360 nm (Cecil Instrument CE505). The preparative TLC plates employed in the quantification were 0.5 µm thick. On detection of the area containing the toxin of interest, it was scrapped off, eluted with chloroform and filtered using Whatman No 1. filter paper. The extract was evaporated to dryness and reconstituted with 3 ml chloroform. Alongside with aflatoxin standard of 20 µg/ml concentration, the absorbance was determined on an ultraviolet spectrophotometer (Cecil Instrument CE505) at a wavelength of 360 nm.

This approach as used by Jonathan and Esho (2010) was calculated as follows:

$$\text{Aflatoxin concentration, C } (\mu g/kg) = \frac{\text{Absorbance of sample} \times \text{Concentration of standard} \times \text{Dilution Factor}}{\text{Absorbance of Standard}}$$

## RESULTS AND DISCUSSION

The total viable bacterial counts of the cocoyam flour samples during storage for four months ranged from $1.6 \times 10^3$ - $4.8 \times 10^5$ cfu/g (Table 1). There was gradual but steady increase in the bacterial count throughout the

**Table 1.** Total viable counts (TVC) of cocoyam flour stored at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

| Period of storage | Total Viable Count | |
|---|---|---|
| | Bacteria (cfu/g) | Fungi (sfu/g) |
| 0 h | Nil | $5.0 \times 10^1$ |
| 1 week | $1.6 \times 10^3$ | $7.0 \times 10^2$ |
| 2 weeks | $3.5 \times 10^3$ | $6.8 \times 10^3$ |
| 1 month | $1.0 \times 10^4$ | $1.5 \times 10^4$ |
| 2 months | $6.0 \times 10^4$ | $1.7 \times 10^4$ |
| 3 months | $9.1 \times 10^4$ | $2.7 \times 10^5$ |
| 4 months | $4.8 \times 10^5$ | $3.8 \times 10^5$ |

Each value is the mean of triplicate determinations.

**Table 2.** Bacteria isolated from cocoyam flour during storage at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

| Isolate | Period of Storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 Week | 2 Weeks | 1 Month | 2 Months | 3 Months | 4 Months |
| *Bacillus subtilis* | - | + | + | + | + | + | - |
| *Staphylococcus epidermidis* | - | - | - | + | + | + | + |
| *Staphylococcus saprophyticus* | - | + | + | + | - | - | - |
| *Klebsiella* species | - | + | + | + | + | + | + |
| *Proteus* species | - | + | + | + | + | - | - |
| *Micrococcus luteus* | - | - | - | - | + | + | + |
| *Streptococcus pyogenes* | - | - | - | - | - | + | + |
| *Pseudomonas* species | - | - | - | - | + | + | + |

+ = Present; - = Absent.

of storage.

The total viable fungal counts of cocoyam flour samples ranged from $5.0 \times 10^1$ to $3.8 \times 10^5$ sfu/g. There was also a gradual increase in the fungal counts with increase in storage period (Table 1).

The increase in microbial load might be due to increase in moisture content during storage. These results are in line with Modupe et al. (2016) who showed that a high moisture content has been reported to potentiate biodeteroriation. The cocoyam flour is hygroscopic and can absorb moisture from the environment. Furthermore, the increase in moisture could be attributed to the type of packaging material used. Low density polythene packaging material has the ability to absorb moisture from the environment.

A total of ten bacterial species were isolated from the cocoyam flour sample during the four months of storage (Table 2), they include: *Staphylococcus epidermidis, Staphylococcus saprophytic, Staphylococcus aureus, Micrococcus luteus, Streptococcus pyogenes,* species of *Bacillus, Klebsiella, Pseudomonas* and *Proteus.* While six fungal genera were isolated (Table 3). The fungal isolates include *Aspergillus niger, Aspergillus flavus,* species of *Rhizopus, Fusarium, Penicillium* and *Mucor.* Bacterial species isolated have been associated with food handlers, equipment and raw materials and they play important role in the spoilage of food and some of them (*Staphylococcus aureus*, *S. pyogenes* and *Bacillus cereus*) are pathogenic (Moretro and Langsrud, 2017). The processing of cocoyam flour involves lot of manual handling and this might be one of the sources of contamination.

*S. aureus* grows well in protein and carbohydrate rich foods and it is tolerant to high levels of salt (Moretro and Langsrud, 2017). According to Sachindra et al. (2005), the processing conditions such as drying and heat treatment might reduce microbial levels, but recontamination could take place during the post-processing handling or storage practices. Processing and storage conditions may influence the presence and number of microorganisms present in the processed cocoyam flour. The growth conditions for microorganisms are dependent on specific intrinsic and extrinsic factors such as temperature, water activity, pH, oxidation-reduction potential, microbial interactions and nutrient content (Jay, 2000).

The predominant fungi were *Fusarium* spp., *A. niger* and *A. flavus*. *A. niger, A. flavus* and *Penicillium* spp.

**Table 3.** Fungi associated with cocoyam flour during storage at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

| Isolate | Period of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 Week | 2 Weeks | 1 Month | 2 Months | 3 Months | 4 Months |
| *Rhizopus* species | - | + | + | + | + | + | + |
| *Aspergillus niger* | + | - | - | + | + | + | + |
| *Aspergillus flavus* | + | + | + | + | + | + | + |
| *Fusarium* species | + | + | + | + | + | + | + |
| *Penicillium* species | + | + | + | + | + | + | + |
| *Mucor* species | - | - | - | - | + | + | + |

+ = Present; - = Absent.

**Table 4.** Biochemical (Nutritional) quality of cocoyam flour stored at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

| Parameter | % Composition | |
|---|---|---|
| | 0 h | 4th Month |
| Moisture | 5.27 ± 0.01 | 6.90 ± 0.02 |
| Ash | 3.28 ± 0.04 | 2.92 ± 0.03 |
| Protein | 15.84 ± 0.02 | 14.95 ± 0.01 |
| Fat | 1.93 ± 0.05 | 1.86 ± 0.02 |
| Carbohydrate | 67.76 ± 0.003 | 58.08 ± 0.005 |
| Crude Fibre | 0.76 ± 0.01 | 0.67 ± 0.04 |

Each value is the mean ± standard deviation of triplicate determinations.

were isolated throughout the storage of the flour samples. *Mucor* spp. emerged after two months of storage. This indicated that ecological succession may have occurred during the storage of the cocoyam flour.

High number of fungi in the final product may indicate poor handling during processing and storage conditions (temperature and humidity) which allowed the growth and proliferation of these organisms (Mandeel, 2005).

Fungi are widely distributed in air and in the soil (Braide et al., 2008). *Aspergillus* and *Penicillium* spp. are frequently isolated from food and may have contaminated the products through the soil during processing and storage (Abbey, 2007). *Rhizopus* and *Mucor* spp. are less fastidious and are frequently involved in the deterioration and spoilage of food with low moisture content (Braide et al., 2011).

There was notable increase in the moisture content (5.27%-14.90%) at the end of storage period as presented in Table 4. However, decreases were observed in percentage Ash (3.28 - 2.92%), Protein (15.84 - 14.95%), Fats (1.93 - 1.86%), Crude Fibre (0.76 - 0.67%) and Carbohydrates (67.76 - 58.08%) contents at the end of four months.

The decrease in carbohydrate, protein and fat could be attributed to high microbial activities potentiated by the high moisture content. Also, the drying process applied may have denatured the protein structure leading to decrease with storage period. Presence of microbial contaminants may have encouraged utilization of the nutrients in the stored cocoyam flour for their growth and proliferation. Increase in moisture content could also be attributed to the low density polythene packaging material which has the ability to absorb moisture from the environment. High moisture content encourages prolific growth of bacteria and mould in foods (Kordylas, 1991).

There was a notable decrease in pH (6.40 - 4.17) and increase in percentages TA (0.024 - 1.116%) during storage period (Table 5). The low pH observed may be related to the activities of associated microbes which may have increased the release of some organic acids and other metabolites; thereby increasing the titratable acidity.

The mean aflatoxin concentration of the sample obtained is shown in Table 6. Aflatoxin G1 and G2 were not detected in the samples analyzed at 0 h, but B1 and B2 were detected and also as the month of storage increased, the level of aflatoxin content gradually increased. *Aspergillus, Penicillium* and *Fusarium* spp. produce various mycotoxins in food under storage (Efiuvwevwere, 2000; Abbey, 2007). *A. flavus* elaborate aflatoxins that may induce hepatocellular carcinoma.

Toxins produced by *Penicillium* spp. may be nephrotoxic and carcinogenic, *Fusarium* spp. toxins give

**Table 5.** Physico-chemical quality of packaged cocoyam flour stored at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

| Period of storage | pH | TA (as % Lactic acid) |
|---|---|---|
| 0 h | 6.40 ± 0.001 | 0.024 ± 0.003 |
| 1 Week | 5.97 ± 0.03 | 0.09 ± 0.001 |
| 2 Weeks | 5.70 ± 0.01 | 0.109 ± 0.003 |
| 1 Month | 5.60 ± 0.02 | 0.128 ± 0.05 |
| 2 Months | 5.07 ± 0.04 | 0.162 ± 0.03 |
| 3 Months | 4.50 ± 0.03 | 0.17 ± 0.01 |
| 4 Months | 4.17 ± 0.01 | 1.17 ± 0.01 |

Each value is the mean ± standard deviation of triplicate determinations.

**Table 6.** Aflatoxin content (µg/kg) in samples of packaged cocoyam flour stored at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

| Period of storage | Aflatoxin content (µg/kg) | | | |
|---|---|---|---|---|
| | $B_1$ | $B_2$ | $G_1$ | $G_2$ |
| 0 h | 0.020 | 0.006 | 0.00 | 0.00 |
| 1 month | 0.041 | 0.029 | 0.011 | 0.009 |
| 2 months | 0.046 | 0.033 | 0.017 | 0.010 |
| 3 months | 0.078 | 0.057 | 0.026 | 0.019 |
| 4 months | 0.097 | 0.063 | 0.031 | 0.025 |

rise to allergic symptoms or are carcinogenic in long term consumption (Pitt, 2000; Abbey, 2007). *Rhizopus* and *Mucor* spp. also produce mycotoxins associated with various mycotoxicoses (Abbey, 2007). The presence of mycotoxins in our food systems and tissues has enormous public health significance because these toxins are nephrotoxic, immunotoxic, teratogenic and mutagenic. They are also capable of causing acute and chronic effects in man and animals ranging from death to disorder of central nervous, cardiovascular, pulmonary systems and intestinal tract (Bhat and Vasanthi, 2003).

The values of aflatoxin determined were insignificant when compared with the values provided by National Agency for Food and Drug Adminstration and Contol (NAFDAC), Nigeria. NAFDAC has recently given a permissible limit for AFB1 of 4 - 5µg for beans, wheat and flours (Makun et. al., 2010).

## Conclusion

The study has revealed that obvious microbiological, physico-chemical and biochemical changes took place during processing and storage of cocoyam flour packaged in low-density polyethylene (LDPE) under tropical temperature. The presence of aflatoxin $B_1$ and $B_2$ in the stored product is of public health concern. Therefore, there is need for improved processing and handling techniques, hygiene practices and safety of the finished product. Findings obtained may be useful in the handling and storage of cocoyam flour.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES


Abbey SD (2007). Foundation in Medical Mycology, 4th edition Kenalf Publication, Port Harcourt, Nigeria pp. 22-30.

Adekiya AO, Ojemji SO, Agbede TM, Ewulo BS (2014). Soil physical properties and cocoyam performance as influenced by tillage on an Affisol in the rain forest zones of South West Nigeria. International soil tillage research organization pp. 266-271.

Association of Official Analytical Chemists (AOAC) (2008).Official method of analysis, Washington D.C association of Official analytical chemists, 17th edition.

Bhat RV, Vasanthi S (2003). Food Safety in Food Security and Food Trade. Mycotoxin. Food Safety Risk in Developing Countries. International Food Policy Research Institute.

Boakye AA, Wireko-Manu FD, Oduro I, Ellis WO, Gudjónsdóttir M, Chronakis IS (2018). Utilizing cocoyam (*Xanthosoma sagittifolium*) for food and nutrition security: A review. Food Science Nutrition 6(4):703-713.

Braide W, Sokari TG, Nwaoguikpe RN, Okorondu SI (2008). Microbes from soils associated with metamorphosing moth larvae. Microbial Technique 4:11-14.

Braide W, Nwaoguikpe R, Udegbunam I, Akobondu C, Okorondu S Solomon O (2011). The effect of biodeterioration on the nutritional composition and microbiology of an edible long-winged reproductive termite, *Macroterms bellicosus*. Smeathman. Internet Journal of Food Safety 13:107-114.

Chukwu GO, Nwosu KI, Mbanaso ENA, Onwubiko O, Oloye BC (2012). Cocoyam rebirth initiative. Annual Report National Root Crops Research Institute (NRCRI). Umudike pp. 119-121.

Cole P (2002). Determining moisture in food. http://www.foodtechsource.com (Accessed 16/4/2013)

Efiuvwevwere BJO (2000). Microbial Spoilage agents of Tropical and Assorted Fruits and Vegetables: An Illustrative Reference Book. First edition, Published by Paragraphics, Port Harcourt, Nigeria pp. 3-8.

Food Agricultural Organization (FAO) (2012). Root and tuber crops in developing countries, challenges and opportunities pp. 11-13.

Ejoh SI, Obatolu VA, Olanipekun OT, Farinde EO (2013). Extending the use of an underutilized tuber physicochemical and pasting properties of Cocoyam (*Xanthosoma safittifolium)* flour and it's suitability for making biscuit. African Journal of food Science 7:264-273.

Jay JM (2000). Modern Food Microbiology. Aspen Publishers, Inc. Gaithersburg, Maryland pp. 35-41, 388-395.

Jonathan SG, Esho EO (2010). Fungi and Aflatoxin detection in two oyster mushrooms *Pleurotus ostreatus* and *Pleurotus pulmonanus* from Nigeria. Electronic Journal of Environmental Agricultural and Food Chemistry 11:1722-1730.

Kordylas JM (1991). Processing and Preservation of Tropical and Subtropical Foods, 1st Edition Macmillian Education Ltd., Baskingstoke 414 p.

Kundu N, Campbell P, Hampton A (2012). Anti - metastatic activity isolated from *Colocasia esculenta* (Taro). Anti-cancer drugs pp. 200-211.

Makun HA, Anjorin ST, Moronfoye B, Adejo FO, Afolabi OA, Fagbayibo G, Balogun BO, Surajudeen AA (2010). Fungal and aflatoxin contamination of some human food commodities in Nigeria. African Journal of. Food Science 4(4):127-135.

Mandeel QA (2005). Fungal contamination of some imported spices. Mycopathologia 159(2):291-298.

Modupe EO, Abiodun JO, Adesola AA (2016). The study of the effect of moisture content on the Biochemical deterioration of stored fermented *Parkia biglobosa* seeds. Open Journal of Engineering Research and Technology 1(1):14-22.

Moretro T, Langsrud S (2017). Residential Bacteria on Surfaces in the Food Industry and Their Implications for Food Safety and Quality. Comprehensive Reviews in Food Science and Food Safety 16(5):759-1169.

Nnabuk OE, Emmanuel E, Eno EE, Richard AU (2012). Industrial Potential of Two Varieties of Cocoyam in Bread Making. E-Journal of Chemistry 9(1):451-464.

Ochei J, Kolhatkar A (2008). Medical laboratory science. Theory and practice. McGraw-Hill, New York, USA.

Olajide R, Akinsoyinu AO, Babayemi OJ, Omojoja AB, Abu AO, Afolabi KD (2011). Effect of processing on energy value, nutrient and anti-nutrient components of wild Cocoyam (*Colocosia esculenta)*, Pakistan journal of nutrition 10(1):29-34.

Onwubuya EA, Ajani EN (2012). Strategies for improving production and processing of cocoyam among women in Anambra State, Nigeria. Universal Journal of Education and General Studies 1(16):169-173.

Onyeka J (2014). Status of Cocoyam (*Colocasia esculenta* and *Xanthosoma* spp.) in West and Central Africa : Production, Household Importance and the Threat from Leaf Blight. Technical report.

Pitt JI (2000). Toxigenic Fungi: Which are important? Medical Mycology 38(1):17-22.

Sachindra NM, Sakhare PZ, Yashoda KP, Narasimha RD (2005). Microbial profile of buffalo sausage during processing and storage. Food Control 16(1):31-35.

Sefa-Dede S, Sackey EKA (2002). Starch structure and some properties of cocoyam (*Xanthosoma saggitifolium* and *Colocasia esculenta*) starch and raphides. Food Chemistry 79(4):435-444.

Tagodoe A, Nip WK (1994). Functional properties of raw and precooked taro *(Colocasia esculenta*) flours. International Journal of Food Science Technology 29(4):457-462.

Vol. 15(6), pp. 278-285, June, 2021
DOI: 10.5897/AJMR2021.9502
Article Number: 2A7C82D66931
ISSN: 1996-0808
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African Journal of Microbiology Research

*Full Length Research Paper*

# Monitoring of physicochemical and microbiological parameters during *nonnonkoumou* (artisanal curdled milk) production in Daloa, Côte d'Ivoire

**ASSOHOUN-DJENI Nanouman Marina Christelle[1*], ATTIEN Yao Paul[1], KOUASSI Kra Athanase[1], KOUASSI Kouassi Clément[1], KOUAME Aya Bah Marie-Ange Christelle[1], DJENI N'dédé Théodore[2] and KONATE Ibrahim[1]**

[1]Université Jean Lorougnon Guédé, Unité de Formation et de Recherche en Agroforesterie, Laboratoire de microbiologie, Bio-industrie et Biotechnologie, BP150 Daloa, Côte d'Ivoire.
[2]Université Nangui Abrogoua, Unité de Formation et de Recherche en Sciences et Technologies des Aliments, Laboratoire de Biotechnologie et microbiologie des aliments, 02 BP 801 Abidjan 02 Côte d'Ivoire.

Received 15 February, 2021; Accepted 31 March, 2021

**This study aimed to study physicochemical and microbiological parameters during *nonnonkoumou* production. A total of 15 samples were analyzed during this study at a rate of 3 samples per fermentation time ($T_{0\ h}$, $T_{6\ h}$, $T_{12\ h}$, $T_{18\ h}$ and $T_{24\ h}$). The physicochemical analyses showed a drop in pH (from 7.03 ± 0.028 to 4.59 ± 0.021) during the 24 h of fermentation. The titratable acidity increased from 0.30 ± 0.014% at the start of fermentation to reach the maximum value of 0.88 ± 0.056% at 24 h of fermentation. The sugar level of fermenting milk samples decreased from 10.05 ± 0.071% at the start of fermentation, to 5.15 ± 0.071% at the end of fermentation. The density results showed an addition of water to the milk used for *nonnonkoumou* production. Microbiological analyses showed a similar growth of lactic acid bacteria and yeasts and molds, but yeasts and molds were absent at the start of fermentation. Aerobic mesophile flora count reached maximum value (7.56 ± 0.81 Log CFU/ml) at 12 h of fermentation. The coliform count increased up to 12 h of fermentation before decreasing and disappearing at the end of fermentation.**

**Key words:** Milk, *nonnonkoumou,* fermentation, contamination, physicochemical and microbiological analyses.

## INTRODUCTION

Milk is an edible biological liquid, usually whitish in color, produced by the mammary glands of female mammals. Rich in lactose, it is the main source of nutrients for young mammals before they can digest other types of food (FAO, 2011). Like dairy products, milk is a balanced food because it provides the human body with around 15 essential elements for the maintenance of good health (Das et al., 2015). Milk provides relatively quick incomes

*Corresponding author E-mail: ass_marina@yahoo.fr.

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for small producers and is an important source of income (FAO, 2011). Among farmed products (skins for the preparation of leather products, meat), milk occupies an important place in African economies. In addition, milk represents a biological medium that is highly alterable by microbial means because of its high water content, its pH close to its neutrality and its richness in biodegradable components (lactose, proteins and lipids). When taken under the right conditions (Aseptic conditions), raw milk contains few bacteria ($10^3$ germs/ml). These are saprophytic bacteria and among them are the lactic streptococci (*Lactococcus*) and lactobacilli. During milking and storage, the milk can become contaminated with a variety of microflora consisting of essentially of lactic acid bacteria belonging to the following genera: *Streptococcus, Lactococcus, Enterococcus, Leuconostocs* and *Lactobacillus* (Bekhouche, 2006). Milk can also be contaminated by contaminating microorganisms (total coliforms, thermotolerant coliforms), and even pathogenic bacteria such as *Salmonella* (Assohoun et al., 2020). The high temperatures in some African countries are responsible of the quick degradation of cow milk; therefore, techniques are needed for processing milk to extend shelf life including the preparation of cheeses. Cow milk was once entirely self-consumed but is now transformed into the artisanal curdled products such as *Rayeb* and *Jben* in North Africa, *Bouhazza* in some rural areas in eastern Algeria, *Iben* in Moroccan (Kèkè et al., 2009), and also *nonnonkoumou* in Côte d'Ivoire. *Nonnonkoumou* is an artisanal curdled milk, consumed in Côte d'Ivoire and particularly in Daloa city. Its production is done in a traditional way without the use of starter cultures. This is a spontaneous fermentation initiated by natural microorganisms that are found in the milk, on the processing utensils/equipment, on the hands of producers and from the local atmosphere as natural starters (Assohoun et al., 2012). The result is a heterogeneous end product, with varying characteristics from one producer to another. The pH of the milk which is around the neutrality is very low in the *nonnonkoumou* (pH around 4) (Assohoun-Djeni et al., 2020). This variation in pH is associated with other parameters (titratable acidity rate, density, etc.) during milk fermentation for *nonnonkoumou* production and is associated with the growth of certain microorganisms such as lactic acid bacteria which produce lactic acid, hydrogen peroxide and bacteriocins. These metabolites influence the growth of certain microorganisms present in fermenting milk. To our knowledge, very few studies have been carried out on the fermentation process of milk used for *nonnonkoumou* production. In order to enhance the *nonnonkoumou* by mastering the manufacturing process, it would be interesting to deepen the understanding on the fermentation of milk in order to guarantee a stable and quality product at the end of production.

The objective of this work was to study the evolution of some physicochemical and microbiological parameters during the milk fermentation for *nonnonkoumou* production in Daloa.

## MATERIALS AND METHODS

### Sampling

The samples analyzed during this study consisted of fermenting milk collected taken at different times ($T_0$, $T_{6H}$, $T_{12H}$, $T_{18H}$ and $T_{24H}$) during fermentation of milk. Indeed, 1 L of milk is taken from 3 resellers and boiled for about 10 min. The milk is then cooled and then distributed in 5 plastic bottles and fermented in a container after packing in a cloth as the producers do. Every 6 h, a bottle containing the fermenting milk is removed and the contents analyzed. Fermentation is thus carried out for 24 h. In total, 15 samples were taken and analyzed during the present study at a rate of 3 samples per fermentation time. Each sample consisted of approximately 200 ml of *nonnonkoumou.*

### Physico-chemical analysis

#### *pH determination*

The pH of fermenting milk was determined using a pH meter (Microprocessor pH meter, pH 211, HANNA Instruments) according to the AOAC (1995) method. The instrument was calibrated using two buffer solutions at pH 7.0 and 4.0 and this was systematically done before pH measuring. The measurement was made by immersing the electrode in 20 mL of sample and the reading is repeated three times.

#### *Total titratable acidity (TA) determination*

Total titratable acidity (TA) was measured by titrating 10 mL of fermenting milk against 0.1N sodium hydroxide (NaOH) solution using phenolphthalein as indicator (Kimaryo et al., 2000) and the reading is repeated three times. The lactic acid level (corresponding to the level of titratable acidity) was determined by the following expression:

$$\text{Acid level (\%)} = \frac{\text{Vol (NaOH)} \times \text{N (NaOH)} \times 0.09}{\text{Quantity taken for test}} \times 100$$

where Vol (NaOH) = NaOH volume (in ml) used for the determination, N (NaOH) = normality of NaOH solution, quantity taken for test = 10 mL, and 0.09 = factor (in mill equivalent) for lactic acid.

#### Density determination

To deduce the fraudulent practices of sellers by adding external elements, the density of the samples of fermentation milk was determined according to formula used by Pointurier (2003):

d = ml / me

where d = density of milk at 20°C, ml = mass of 10 ml of milk, and me = mass of 10 ml of tap water.

When milk temperature at the measurement time is above 20°C, the calculated density is increased by 0.0002 per degree above 20°C. When the temperature of the milk at the time of measurement is below 20°C, the calculated density is decreased by 0.0002 per degree below 20°C. Normal milk has a specific density at 20°C between 1.028 and 1.032.

#### Brix degree or refractometric dry extract (E.S.R) determination

The Brix degree or refractometric dry extract (E.S.R) which is the weight (in grams) of soluble dry matter contained in 100 g of product was measured using a refractometer (El Bouichou 2015). So, a drop of fermenting milk was placed on the movable jaw then this jaw was folded back. The reading is carried out in the presence of a light source and the number corresponding to the line of separation of the clear zone and of the blue zone gives the degree Brix or the refractometric dry extract (expressed as a percentage).

#### Enumeration of microorganisms

Preparation of stock solutions, inoculation of agar plates, and cultivation and quantification of microorganisms were carried out according to Coulin et al. (2006). For all determinations, 10 ml of the sample were homogenized in a stomacher with 90 ml of sterile diluent containing 0.85% NaCl and 0.1% peptone (Difco, Becton Dickinson, Sparks, MD, USA). Tenfold serial dilutions of stomacher fluid, ranging from $10^1$ to $10^7$, were prepared and spread-plated for the determination of microbial counts. So, enumeration of coliforms was carried out using violet crystal and neutral red bile lactose (VRBL) plates containing agar (VRBL agar, Oxoid Ltd., Basingstore, UK), incubated for 24 h at 30°C for total coliforms and 44°C for fecal coliforms. Yeasts and molds were enumerated on Sabouraud chloramphenicol agar (Fluka, Biochemica 89579, Sigma–Aldrich Chimie GmbH, India) incubated at 30°C for 4 days. Aerobic mesophiles were enumerated on Plate Count Agar (PCA Oxoid.) and incubated at 30°C for 2 days. Enumeration of LAB was carried out using Man, Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany), which were incubated under anaerobic conditions (Anaerocult A, Merck) at 37°C for 72 h.

#### Colony enumeration

Colony forming units per milliliter of sample (CFU/g) were calculated according to standard NF/ISO 7218: 2007 using the following formula:

$$N = \frac{\sum c}{d(n_1 + 0{,}1 n_2)v}$$

where ΣC: sum of characteristic colonies counted on all retained Petri dishes; $n_1$: number of Petri dishes retained at the first dilution; $n_2$: number of Petri dishes retained at the second dilution; d: dilution rate corresponding to the first dilution; V: inoculated volume (mL); N: number of microorganisms (CFU/g).

#### Statistical analysis

All trials were repeated four times. The different sample treatments were compared by performing one-way analysis of variance on the replicates at a 95% level of significance using the Statistica (99th Ed, Alabama, USA) statistical program. Unless otherwise stated, significant results refer to $P < 0.05$. This software was also used to calculate mean values and standard deviations of the trials.

## RESULTS

### Change of pH, titratable acidity, refractometric dry extract and density

The pH dropped significantly during the *nonnonkoumou* production from 7.03 ± 0.028 at 0 h of fermentation to 4.59 ± 0.021 at 24 h of fermentation contrarily to the total titratable acidity which amount increased at the same time from 0.30 ± 0.014 to 0.88 ± 0.056%. Regarding density, the values are not significantly different at the 5% threshold. With a value of 1.01 ± 0.028 at the start of fermentation (T0 h), the density decreased very slowly and reached the value of 0.98 ± 0.021 at 18 h of fermentation. A slight increase (1.01 ± 0.007) was observed at the end of fermentation (T24 h). As for the refractometric dry extract (E.S.R.), its value is 10.05 ± 0.071% at the start of fermentation. This value decreased during fermentation until it reached its minimum value of 5.15 ± 0.071% at 24 h of fermentation (Table 1).

### Enumeration of the different bacterial groups

Enumeration of microorganisms in this study showed that LAB and yeasts and molds had similar growth throughout the fermentation, but their counts were significantly different ($P < 0.05$) at the different fermentation times (Figure 1). Indeed, LAB mean load was 1.27 ± 0.12 Log CFU/ml at the start of fermentation but yeasts and molds were absent. Yeasts and molds appear after 6 h of fermentation with a load of 3.95 ± 0.32 Log CFU/ml. The LAB and yeasts and molds counts increased during the process and reached highest values (8.56 ± 0.18 Log CFU/ml for LAB and 6.31 ± 0.54 log CFU/g for yeasts and molds) at 18 h of fermentation, before decreasing until the end of fermentation (Figure 1). However, the LAB count stayed significantly higher than that of yeasts and molds. Contrarily to the fermenting microorganisms (LAB and yeasts and molds), the total and fecal coliform populations, with respective initial loads of 4.22 ± 0.52 Log CFU/ml and 3.73 ± 0.45 Log CFU/ml increased slightly to reach their maximum at 12 h of fermentation. After this 12 h of fermentation, these microorganisms decreased rapidly and disappeared after 18 h of fermentation. However, the aerobic mesophiles count was very high (6.22 ± 0.56 Log CFU/ml) at the start of fermentation. This count increased further during the process to reach the value of 7.56 ± 0.81 Log CFU/ml at 12 h of fermentation before experiencing a slight decrease at the end of fermentation (Figure 2).

**Table 1.** Evolution of pH, titratable acidity, refractometric dry extract (ESR) and density of *Nonnonkoumou* samples taken at different fermentation times.

| Fermentation time (h) | Parameter | | | |
|---|---|---|---|---|
| | ESR (%) | Density | Titratable acidity (%) | pH |
| 0 | 10.05±0.071[a] | 1.01±0.028[a] | 0.30±0.014[d] | 7.03±0.028[a] |
| 6 | 9.65±0.071[b] | 1.00±0.028[a] | 0.33±0.035[d] | 6.54±0.014[b] |
| 12 | 6.05±0.071[c] | 0.99±0.021[a] | 0.47±0.028[c] | 6.21±0.021[c] |
| 18 | 5.95±0.071[c] | 0.98±0.021[a] | 0.69±0.007[b] | 5.63±0.028[d] |
| 24 | 5.15±0.071[d] | 1.01±0.007[a] | 0.88±0.056[a] | 4.59±0.021[e] |

ESR: Refractometric dry extract. Values at each time point are the means of our replicates ± SD (error bars). The same letter in the same column indicated no statistical difference (P> 0.05) (Tukey, HSD).

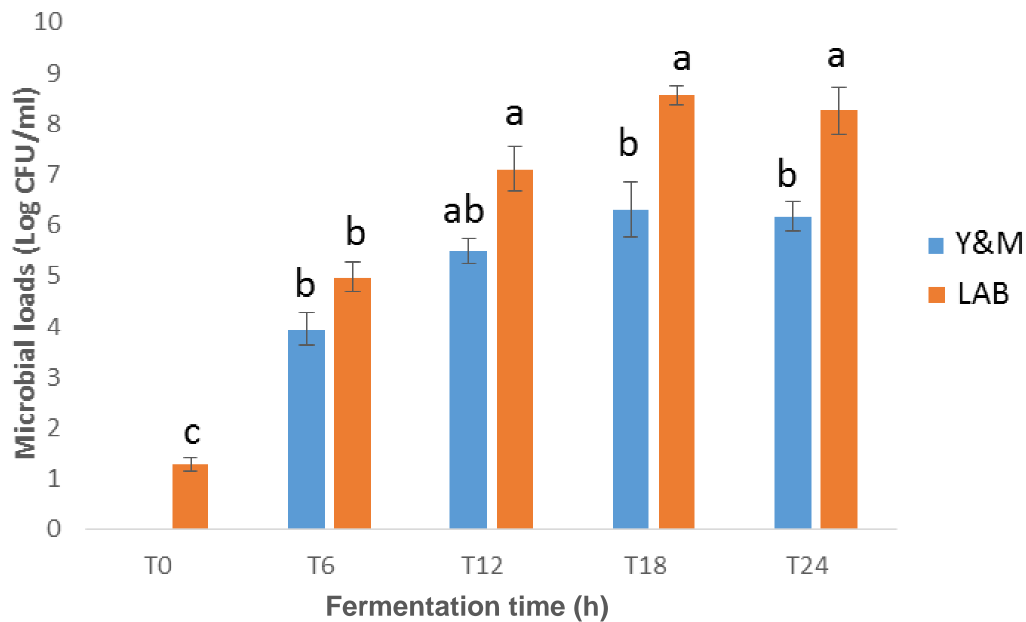


**Figure 1.** Evolution of lactic acid bacteria and yeasts and molds (fermenting microorganisms) populations in *nonnonkoumou* samples taken at different fermentation times. Values at each time point are the means of our replicates ± SD (error bars). For the same type of germ, histograms bearing the same alphabetical letter are not statistically different (P> 0.05) (Tukey, HSD). LAB: Lactic acid bacteria, Y&M: Yeast and molds.

## DISCUSSION

*Nonnonkoumou* is an artisanal curdled milk consumed in Côte d'Ivoire. Its production is done in an artisanal way and its fermentation is spontaneous. The present study focused on the common, fermentative and contaminating microflora of *nonnonkoumou* throughout the manufacturing process. The study also aimed to determine some physicochemical characteristics of the *nonnonkoumou* samples taken at different fermentation times during the production of this food. Acidity is an important quality indicator of fermented milk, which is closely related to the texture and flavor of the product (Li et al., 2017). This is also the case of Moroccan *Iben* which is a dairy beverage prepared by spontaneous fermentation and coagulation of whole raw milk. This dairy product has a low pH (4.2), responsible for the texture of the final product (Tantaoui-Elaraki and El Marrakchi, 1987). Appropriate acidity gives the product a unique flavor and inhibits the growth of spoilage bacteria and food-borne pathogens (Mufandaedza et al., 2006). Changes in pH and titratable acidity are shown in Table 1. Indeed, the pH of the milk at the start of fermentation is 7.03 ± 0.028. There is significant acidification resulting in

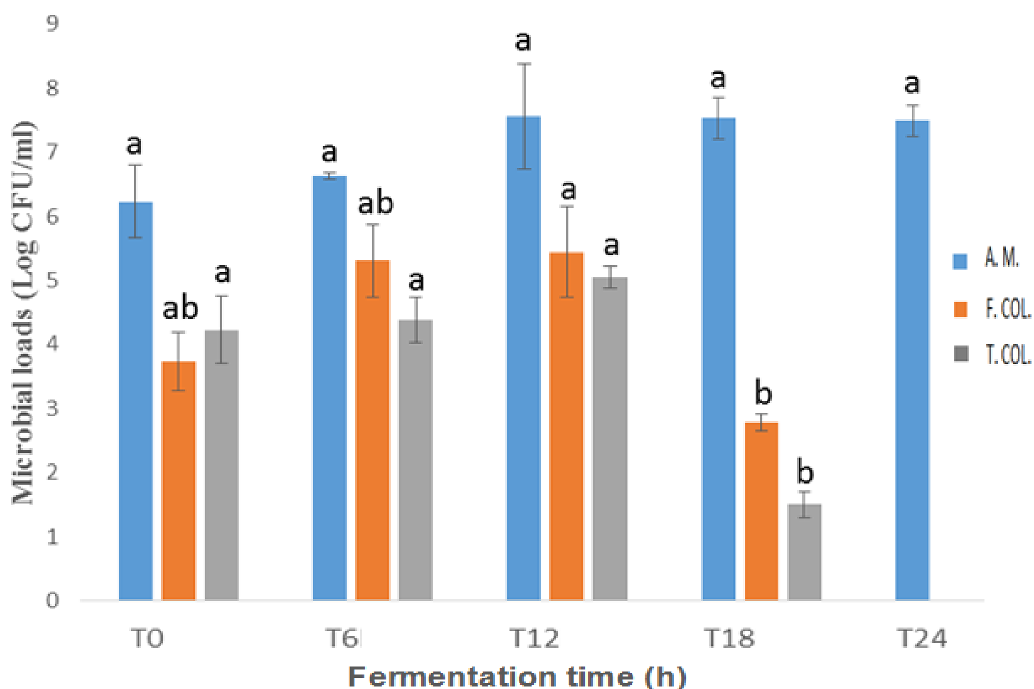


**Figure 2.** Evolution of aerobic mesophiles, total and fecal coliforms populations (non fermenting microorganisms) in *nonnonkoumou* samples taken at different fermentation times. Values at each time point are the means of our replicates ± SD (error bars). For the same type of germ, histograms bearing the same alphabetical letter are not statistically different (P> 0.05) (Tukey, HSD). A.M.: Aerobic mesophiles, T. COL.: Total coliforms, F. COL.: Fecal coliforms.

a considerable drop in pH of around 2.44 units (from 7.03 ± 0.028 to 4.59 ± 0.021 units at the end of fermentation) correlated with an increase in the titratable acidity rate ranging from 0.30 ± 0.014% at 0 h of fermentation to 0.88 ± 0.056% at 24 h of fermentation. The pH values decreased while titratable acidity increased during the period of fermentation of milk mostly due to the accumulation of lactic acid by the metabolism of LAB (Coulin et al., 2006; Panda et al., 2007). These results are in agreement with those of Zhihai et al. (2020) which showed a decrease in pH correlated with an increase in titratable acidity during the fermentation of goat milk in China. Furthermore, there are no significant differences between the densities of the *nonnonkoumou* samples taken at different fermentation times. The *nonnonkoumou* samples density varied from 0.832 ± 0.02 at 0 h of fermentation to 0.860 ± 0.01 at the end of fermentation. According to Pointurier (2003), normal milk has a density at 20°C between 1.028 and 1.032 (Table 1). Thus, a density of less than 1.028 indicates the addition of water in the milk ("wet" milk) while a density greater than 1.032 indicates that the milk has been heated or skimmed. It can therefore deduce that the milk used for the *nonnonkoumou* production during this study, which has a density of less than 1.028, is "wet" milk. In other words, the resellers added water to the milked milk at the pasture before the sale in order to increase their turnover. This practice, unfortunately used fraudulently by several resellers, can influence the quality of the finished curd product which is *nonnonkoumou* in the present study. The density of milk also varies according to the dry matter content and the fat content. So it decreases with the increase in fat (Mahamedi, 2015). Also, the refractometric dry extract (E.S.R.) which is the weight (in grams) of soluble dry matter contained in 100 g of product was measured during this study using the refractometer which allowed the determination of the sugar content of *nonnonkoumou*. Thus, at the start of fermentation, the refractometric dry extract has a value of 10.05 ± 0.071. This value decreases considerably during fermentation until reaching the value of 5.15 ± 0.071 at 24 h of fermentation. In fact, microorganisms, particularly lactic acid bacteria, use the sugars present in the milk for their growth during fermentation. Hence, the considerable drop in the sugar content during the production of this artisanal curdled milk commonly called n*onnonkoumou* in malinke language in Côte d'Ivoire. These results are in agreement with those of O'Mahony (1988) who showed that the lactose content of milk could vary during fermentation. The decrease in the sugar level could also

be due to the addition of water to raw milk before making the curd milk (El Bouichon, 2015). The microbiological parameters of milk and *nonnonkoumou* were also analyzed in this study. Indeed, the results obtained have shown the presence of LAB and yeasts and molds in artisanal curd milk (*nonnonkoumou*). As shown in Figure 1, the total LAB count in *nonnonkoumou* samples increased during fermentation and exceeded the minimum bacteria populations ($10^6$ cfu/mL), required for probiotic foods to possess health claims. Vieira-Dalode et al. (2007) indicated in their work the presence of lactic acid bacteria, yeasts and molds and their importance in fermented products. Also, Zhihai et al. (2020) showed the presence of these microorganisms during the fermentation of goat's milk in China. These fermentative microorganisms are abundant in *nonnonkoumou* samples and present a similar evolution. This is in agreement with the work of Chaves-López et al. (2014), who reported that co-culturing with yeasts was able to promote the multiplication of LAB. This phenomenon might be attributed to the release of amino acids and vitamins through the metabolism of yeasts (Zhang et al., 2017), which might provide more nutrients for the growth of LAB. Unlike contaminating microorganisms, the abundance and maintenance of yeasts, molds and lactic acid bacteria in *nonnonkoumou* would surely also be linked on one hand to their capacity to tolerate high concentrations of organic acids and therefore to low pH, and on the other part, to their ability to use the substrates present in the fermentation medium for their development. Yeasts would also make a useful contribution to the flavor and acceptability of fermented products according to these same authors. Lactic acid bacteria produce several aromatic compounds, enzymes and other compounds that have a profound effect on the texture and taste of dairy products (Ngassam, 2007). Also, the milk collected at 0 h of fermentation was free of yeasts and molds. This absence of yeast and mold in our milk samples is probably due to the pasteurization of the milk collected from the resellers before the start of fermentation. Yeasts and molds are said to be heat-sensitive, that is, they do not tolerate high temperatures at all, hence the extermination of their population in these milk samples at 0 h of fermentation. Other microorganisms (total coliforms, fecal coliforms and aerobic mesophiles) were also present in samples analyzed in this study. Indeed, large aerobic mesophile and coliform loads were detected in the samples collected at different times ($T_0$, $T_{6\ h}$, $T_{12\ h}$, $T_{18\ h}$ and $T_{24\ h}$) of fermentation (Figure 2). These contaminating microorganisms would contaminate the milk during milking under generally unsanitary environmental conditions. The surrounding air, the utensils used to extract milk and water could be the main sources of milk contamination. Chye et al. (2004) indeed indicated that such elements (water, utensils, etc.) are major sources of contamination of milk and dairy products. Also, the non-compliance with the rules of hygiene by the sellers, the transport conditions of milk of the pastures to the sale places, the dust as well as the flies which arise on the ladle serving as a measure during the sale, could also be contamination sources (Assohoun-djeni et al., 2020). Millogo et al. (2018) have demonstrated that containers used during milking and in the manufacture of fermented milk can cause contamination of the milk as well as the final product. In addition, Ndiaye (2002) stated that the milk pH is favorable for microorganism growth, but their number decreased as the fermented milk aged. In addition, total and thermotolerant coliforms were detected in large number in milk and fermenting milk collected during this study. The incidence of coliforms in raw milk has received considerable attention, partly due to their association with contamination of fecal origin and the consequent risk of more pathogenic fecal organisms being present, partly because of the spoilage their growth in milk at ambient temperatures can produce. The presence of coliforms in dairy products is not acceptable by safe food consumption standards. Some of these microorganisms can become highly pathogenic and may cause serious diseases for human. Coliform counts regularly in excess of 2 Log cfu/mL are considered as evidence of unsatisfactory production hygiene (Directive 92/46/EEC, 1992). Sporadic high coliform counts may also be a consequence of unrecognized coliform mastitis, mostly caused by *Escherichia coli* (Torkar and Teger, 2008). Their presence in the samples of the present study is linked to poor hygienic practice during the production of *nonnonkoumou*. On the other hand, these coliforms are completely absent in the *nonnonkoumou* samples collected at the end of fermentation (after 18 h of fermentation). This phenomenon is certainly due to the acidity of this food at the end of fermentation which has a low pH (around 4) (Assohoun et al., 2020). This low pH prevents the growth of most spoilage and pathogenic organisms (Varga, 2007). Such results have already been reported by some authors (Karamoko et al., 2012) in their work on fermented foods. Also, Steinkraus (1996) specified that coliforms do not tolerate low pH. This disappearing of coliforms was certainly due to the sensitivity of these bacteria to the substances produced by LAB. It was proved that LAB exerts antimicrobial action through the production of lactic acids, bacteriocins, diacetyl and hydrogen peroxide.

## Conclusion

The general objective of this study was to study the evolution of some physicochemical and microbiological parameters during the production of *nonnonkoumou* in Daloa city. The results of the physicochemical analyses showed a considerable drop in pH during the entire

fermentation process. This drop in pH led to an acidic end product (*nonnonkoumou*) with a pH between 4 and 5. These results also showed that the fermenting milk samples analyzed during this study had a sugar level that gradually decreased during fermentation. The density results showed an addition of water to the milk used to *nonnonkoumou* production. Furthermore, the results of the microbiological analyses showed a similar growth of lactic acid bacteria and of yeasts and molds, but yeasts and molds were absent at the start of fermentation. These results of microbiological analyses also showed high loads of Aerobie mesophile microflora. As for coliforms, their load increased up to 12 h of fermentation before decreasing and disappearing after 18 h of fermentation.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

This research was made possible through funds provided by The World Academy of Sciences, Italy (N° 19-120 RG/BIO/AF/AC_I), with the important contribution of the microbiology team of Laboratory of Biotechnology and Food Microbiology, Faculty of Food Sciences and Technology, University Nangui Abrogoua, Cote d'Ivoire. The authors gratefully acknowledge Prof. Marcellin Dje Koffi, the director of the laboratory.

## REFERENCES

AOAC (1995). Official methods of analysis 16th Ed. Association of official analytical chemists. Washington DC, USA.

Assohoun NMC, Djéni NT, N'Guessan KF, Koussemon M (2012). Preliminary study on antimicrobial properties of lactic acid bacteria involved in the fermentation of corn dough during *Doklu* processing in Côte d'Ivoire. Food (Global Science Books) 6:65-70.

Assohoun-Djeni NMC, Kouassi KC, Kouassi KA, Yao KC, Djeni NT, Konate I (2020). Physicochemical Characteristics and Microbial Contamination of Raw Milk and Artisanal Milk Curd (*Nonnonkoumou*) in Daloa, Côte D'Ivoire. International Journal of Current Microbiology and Applied Sciences 9(3):2667-2683.

Bekhouche F (2006). Lactic bacteria from raw cow's milk and Pectinolytic microorganisms from black and green olives: 1. Isolation and biochemical identification. 2. Evaluation and Optimization of the production of polygalacturonase enzyme. State doctoral thesis in microbiology and enzymology option: food engineering. University of Mentouri Constantine Institute of Food Nutrition and Technologies.

Chaves-López C, Serio A, Paparella A, Martuscelli M, Corsetti A, Tofalo R, Suzzi G (2014). Impact of microbial cultures on proteolysis and release of bioactive peptides in fermented milk. Food Microbiology 42:117-121.

Chye FY, Abdullah A, Ayob MK (2004). Bacteriological quality and safety of raw milk in Malaysia. Food Microbiology 21:535-541.

Coulin P, Farah Z, Assanvo J, Spillman H, Puhan Z (2006). Characterization of the microflora of attiéké, a fermented cassava product during traditional small-scale production. International Journal of Food Microbiology 106:131-136.

Das S, Hasan A, Parveen S (2015). Evaluation of microbial load and quality of milk and milk based dairy products. Journal of Biosciences 3(1):1-4.

Directive 92/46/EEC (1992). Council Directive 92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk based products. Official J.L 268(1409):1-31. Available at: http://ec.europa.eu/food/fs/sfp/mr/mr03 _en.pdf.

El Bouichon H (2015). Contribution to the evaluation of fraudulent practices in milk upon receipt Gharb Chrarda bni Hsen. Edilivre 137:128-151.

FAO (2011). Milk production and dairy products, Rome-Italy Karamoko D, Djeni NT, N'guessan KF, Bouatenin KMJ-P Dje KM (2012). The biochemical and microbiological quality of palm wine samples produced at different periods during tapping and changes which occured during their storage. *Food Control* 26:504-511.

Kèkè M, Yèhouénou B, De Souza C, Sohounhloué DCK (2009). Evaluation of hygienic and nutritional quality of *peulh* cheese treated by *Sorghum vulgaris (L)* and *Pimenta racemosa* (Miller) extracts. Scientific Study and Research 10(1):29-46.

Kimaryo VM, Massawe GA, Olusapo NA, Holzapfel WM (2000). The use of starter culture in the fermentation of cassava for the production of "Kivunde" a traditional Tanzanian food product. International Journal of Food Microbiology 56:179-190.

Li C, Song J, Kwok LY, Wang J, Dong Y, Yu H Chen Y (2017). Influence of *Lactobacillus plantarum* on yogurt fermentation properties and subsequent changes during post fermentation storage. Journal of Dairy Science 100:2512-2525.

Mahamedi A (2015). Study of the hygienic, physicochemical and microbiological qualities of ferments and translational butters intended for communication in various regions of Algeria. Doctoral thesis, Oran University. Algeria. P 16.

Millogo V, Sissao M, Ouédraogo GA (2018). Nutritional and bacteriological quality of samples of some local dairy products in the production chain in Burkina Faso. International Journal of Biological and Chemical Sciences 12(1):244-252.

Mufandaedza J, Viljoen BC, Feresu SB, Gadaga TH (2006). Antimicrobial properties of lactic acid bacteria and yeast-LAB cultures isolated from traditional fermented milk against pathogenic *Escherichia coli* and *Salmonella enteritidis* strains. International Journal of Food Microbiology 108:147-152.

Ndiaye M (2002). Contribution to the study of compliance of the labeling of the microbiological quality of yogurts marketed in Dakar. Doctoral thesis, Cheikh AntaDiop University of Dakar, Dakar P. 11.

Ngassam TC (2007). Characterization of the lactic flora of artisanal fermented milks in Senegal: case of the Niaye zone. Doctoral thesis, Cheikh Anta Diop University of Dakar (Senegal). P 109.

O'Mahony F (1988). Rural dairy technology: Experiences in Ethiopia. ILCA Manual No. 4, Dairy Technology Unit, pp. 3, 8, International Livestock Centre for Africa, Addis Ababa, Ethiopia.

Panda SH, Parmanick M, Ray RC (2007). Lactic acid fermentation of sweet potato (*Ipomoea batatas L.*) into pickles. Journal of food processing and Preservation 31:83-101.

Pointurier H (2003). Materials management in the dairy industry. Tec and Doc, Lavoisier, France. P 388.

Steinkraus KH (1996). Handbook of Indigenous Fermented Foods. 2nd edn. Marcel Dekker, New York 1996, ISBN 0 82479 352 8. P 792.

Tantaoui-Elaraki A, El Marrakchi A (1987). Study of Moroccan dairy products: Iben and Smen Journal of Applied Microbiology and Biotechnology 3:211-220.

Torkar KG, Teger SG (2008). The microbiological quality of raw milk after introducing the two day's milk collecting system. Acta Agriculturea Slovenica 92(1):61-74.

Varga L (2007). Microbiological quality of commercial dairy products. Communicating Current Research and Educational Topics and Trends in *Applied Microbiology*, A. Méndez Vilas (Ed.). pp. 487-494

Vieira-Dalode G, Jespersen L, Hounhouligan J, Moller PL, Nago CM, Jakobsen M (2007). Lactic acid bacteria and yeasts associated with

*gowe* production from sorghum in Benin. Journal of Applied Microbiology 103(2):342-349.

Zhang DD, Liu JL, Jiang TM, Li L, Fang GZ, Liu YP, Chen LJ (2017). Influence of *Kluyveromyces marxianus* on proteins, peptides, and amino acids in Lactobacillus-fermented milk. Food Science Biotechnology 26(3):739-748.

Zhihai H, Lu H, Guangliang X, Xiao X, Chuanhai T, Mingsheng D (2020). Effect of Co-Fermentation with Lactic Acid Bacteria and *K. marxianus* on Physicochemical and Sensory Properties of Goat Milk. Foods 9(3):1-14.

Vol. 15(6), pp. 286-294, June, 2021
DOI: 10.5897/AJMR2021.9497
Article Number: 53DF9E666937
ISSN: 1996-0808
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*Full Length Research Paper*

# Molecular characterization of carbapenem resistant Gram-negative rods in Neonatal Intensive Care Unit of Mansoura University Children's Hospital

**Heba Elsayed Eldegla[1,2], Islam Nour[3,4], Nehad Nasef [3,4], Basma Shouman[3,4], Hesham Abdel-Hady[3,4], Mayada Zeid[4,5] , Nawal S. Gouda[1,2*], Manal M. Nomir[6], Abd Elazeez Shabaan[3,4] and Noha Mostafa Mahmoud[1,2]**

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[2]Microbiology Diagnostics and Infection Control Unit, Mansoura University Hospitals, Mansoura, Egypt.
[3]Neonatal Intensive Care Unit, Mansoura University Children's Hospital, Mansoura, Egypt.
[4]Departement of Pediatrics, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[5]Malnutrition and Infectious Diseases Unit, Mansoura University Children's Hospital, Mansoura, Egypt.
[6]Clinical Pathology, Mansoura University Student Hospital, Mansoura, Egypt.

Received 7 February, 2021; Accepted 13 May, 2021

**Carbapenems are group of extended-spectrum β-lactam antimicrobials frequently used for treating multidrug-resistant Gram-negative bacilli (GNB) infections. This study aimed at detecting and characterizing carbapenem resistance (CR) genes among GNB isolated from patients treated in neonatal intensive care unit (NICU) of Mansoura University Children's Hospital (MUCH), Egypt. It is a prospective study conducted from 2015 to 2016. A total of 158 GNB isolates were examined for CR both phenotypically and genotypically. Among 158 Gram negative isolates, there were 58 (36.7%) CR strains. Extended-spectrum β-lactamase (ESBL) production was confirmed in all 58 (100%) isolates. Carbapenemase production was detected in 52 (89.5%) strains while metallo beta-lactamase (MBL) production was found in 33 (56.9%) strains. Molecular characterization of CR strains revealed that 57 (98.3%) isolates were positive for carbapenemase encoding genes. KPC gene was the most frequent detected gene (34/58). VIM, IPM, OXA and NDM genes were also detected in 15, 13, 9 and 1 isolate, respectively. Only one isolate was negative for all encoding resistance genes despite positive for ESBL phenotype. Infection with CR strains has been increasing in clinical settings which limit the use of carbapenems.**

**Key words:** Gram-negative bacilli, carbapenem resistance, carbapenemase, metallo beta-lactamase, multiplex polymerase chain reaction (PCR), carbapenemase encoding genes, neonatal intensive care unit.

## INTRODUCTION

Treating neonatal infections is challenging due to spread of multidrug-resistant bacteria (Mzimela et al., 2021). Carbapenems are final antimicrobial therapy for life threatening microbes, nevertheless, the appearance of carbapenemases in Gram negative bacteria has put the clinicians in front of restricted treatment choices

(Choudhury et al., 2018). Carbapenem resistance (CR) may be due to either carbapenemase production or other mechanisms, such as alteration of outer membrane permeability together with extended-spectrum β-lactamase (ESBL) production, over expression of *AmpC* type β-lactamases or activation of efflux pumps. The production of carbapenemases is generally a more potent mechanism of CR compared with the other mechanisms (Nordmann et al., 2012; Woodford et al., 2004).

Ambler classification categorizes the β-lactamases into four classes (A to D). Most of carbapenemase-producing bacteria related to A class (*bla* $_{KPC}$), B class (*bla* $_{VIM,\ IMP,}$ and $_{NDM}$), and D class (*bla* $_{OXA\text{-}48}$) Albiger et al., 2015). The *Pseudomonas* and *Acinetobacter* species releasing these enzymes have a wide geographical distribution and have been associated with hospital outbreaks (Woodford et al., 2004). Dissemination of carbapenemases is rapid and widespread in healthcare settings (Elbadawi et al., 2021).

Several methods for detecting carbapenemase production have been used. These methods include phenotypic and molecular methods (Al-Zahrani, 2018). This study aimed at molecular characterization of beta-lactamases associated with CR Gram negative neonatal infections.

## METHODOLOGY

### Study design

This prospective study was conducted over 2 years during 2015 and 2016 in the NICU of MUCH, which is a level III unit with 25 incubators in 5 equal-sized rooms, that admits approximately 450 neonates per/year; there are no single rooms for isolation.

### Sample collection and processing

A total of 158 GNB were detected from clinical samples (blood, urine, wound, tracheal aspirate, abscess, etc.) of 350 admitted neonates. Collected specimens were sent to Microbiology Diagnostics and Infection Control Unit laboratory in less than 2 h. If delay in transportation is expected, the specimens (except blood culture bottles) were kept at 4°C in the refrigerator. Clinical samples were processed using the standard laboratory techniques. Microscopic examination of Gram-stained films of the different samples was carried out to find any bacterial cells. Followed by culture on Nutrient agar, Blood agar, MacConkey agar and CLED agar (for urine samples) plates (Oxoid, UK) using the streak plate technique. Plates were kept in incubator at 37°C for 24 h.

### Bacterial identification and susceptibility testing

Identification of the bacterial isolates was performed according to standard procedures, in reference to Mahon et al. (2007/2008). GNB were identified according to colonial appearance, microscopic evaluation and biochemical reactions including Oxidase test using Oxidase strips (Oxoid, UK), Kligler iron agar (KIA) test, Lysine iron agar (LIA) test, Motility, Ornithine production, Indole (MIO) test and Citrate utilization test (Oxoid, UK).

Antimicrobial sensitivity testing was performed by the disc diffusion test using Mueller Hinton (MH) agar (BBL, Becton Dickinson, Cockeysville, MA, USA) and CLSI 2017 M100-S27 breakpoint values were used (CLSI, 2017). Sensitivity testing was performed for the following 10 antibiotics agents: Amoxycillin clavulanate (AMC: 30 µg); Cefuroxime (CXM: 30 µg); Piperacillin-tazobactam (TPZ: 110 µg); Cefoxitin (FOX: 30 µg); Cefipime (FEP: 30 µg); Ceftriaxone (CRO: 30 µg); Ceftazidime (CAZ: 30 µg); Cefotaxime (CTX: 30 µg); Imipenem (IPM: 10 µg), and Meropenem (MEM: 10 µg). Bacterial isolates were diagnosed as CR if they were resistant (diameter ≤ 19 mm) to at least one of the used carbapenems (IPM and MEM).

### Phenotypic detection of carbapenemase activity and ESBL production

The production of ESBL, carbapenemase and MBLs were tested using cephalosporin/clavulanic acid (BD Diagnostics, Franklin Lakes, NJ, USA) combination disc, The Modified Hodge test (MHT) and synergy combined disc test (CDT), respectively.

Phenotypic ESBL production was detected with the combination disc diffusion test with clavulanic acid. The inhibition zone surrounding the cephalosporin (Cefotaxime, Ceftazidime and Cefepime) discs combined with clavulanic acid is compared to the zone around the disc with the cephalosporin alone. The reaction was positive if the inhibition zone was 5 mm larger with clavulanic acid than without (Al Naiemi et al., 2012).

#### *The Modified Hodge test*

a 1:10 dilution of the *Escherichia coli* ATCC 25922 (NAMRU-3 Institute, Naval Medical Research Unit Three, Cairo, Egypt) was made by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of saline. This dilution was streaked on the surface of MH agar plates using a swab, and allowed to dry for 3 to 5 min. Thereafter, 10 µg imipenem disc was placed in the center of the test plate. The test organism was streaked in a straight line from the disk to the edge of the plate. Plates were Kept at 35°C for 24 h. Plates were examined for a clover leaf like indentation of the test isolate and the reference strain of *E. coli* within the zone of inhibition of the imipenem disc (Tamma and Simner, 2018).

#### *Detection of MBL (class B)*

CDT was performed according to Joji et al. (2019). Two 10 µg imipenem discs and two 30 µg ceftazidime discs (Becton Dickinson) were put on a plate inoculated with the test bacteria. 10 µL of sterilized 0.5 M EDTA solution (dissolve 186.1 g disodium EDTA in 1000 ml distilled water at pH 8.0) was supplemented to one disc of each antibiotic. After that, inhibition zones of the imipenem and imipenem + EDTA and ceftazidime and ceftazidime + EDTA discs were compared after 18 to 24 h of incubation at 35°C. A zone diameter difference between the imipenem and imipenem + EDTA discs or the ceftazidime and ceftazidime + EDTA discs ≥7 mm was

*Corresponding author. E. mail: nawalsalama@gmail.com. Tel: 00966502933179.

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**Table 1.** Oligonucleotide primers.

| Gene | Primer sequence (5´→3´) | Amplicon size (bp) |
|---|---|---|
| *bla* $_{KPC}$ | Forward: CGTCTAGTTCTGCTGTCTTG<br>Reverse: CTTGTCATCCTTGTTAGGCG | 798 |
| *bla* $_{VIM}$ | Forward: GATGGTGTTTGGTCGCATA<br>Reverse: CGAATGCGCAGCACCAG | 390 |
| *Bla* $_{IMP}$ | Forward: GGAATAGAGTGGCTTAAYTCT<br>Reverse: CGGTTTAAYAAAACAACCACC | 232 |
| *bla* $_{OXA-48}$ | Forward: GCGTGGTTAAGGATGAACAC<br>Reverse: CATCAAGTTCAACCCAACCG | 438 |
| *bla* $_{NDM-1}$ | Forward: GGTTTGGCGATCTGGTTTTC<br>Reverse: CGGAATGGCTCATCACGATC | 621 |

defined as a positive result for MBL production.

#### Molecular characterization of carbapenemase encoding genes

##### *DNA template*

Genomic DNA was extracted using Gene JET DNA Purification Kit (Thermo Scientific, For 50 preps, Lot. 00189391, The European Union (EU) Lithuania) following the manufacturer instructions.

##### *Multiplex PCR method*

The primers used in this study were based on primers published by Poirel et al. (2011) and are listed in Table 1. The amplification of DNA and thermal cycling conditions were done as described by Karuniawati et al. (2013). One multiplex PCR reaction was done detecting *bla*$_{IMP}$ and *bla*$_{VIM-2}$ genes. Second multiplex PCR reaction was done detecting *bla* $_{KPC}$ and *bla* $_{OXA-48}$ genes. Third reaction was done for detecting *bla*$_{NDM-1}$ gene. DNA amplification was performed through a 50 µl reaction mix having 10 mM Tris-HCL, 50 mM KCL, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 mM of each of the deoxynucleoside triphosphate, 1U of DNA polymerase, 5 µl of template DNA, and 0.4 mM of each primer.

The thermal cycler program was adjusted by using PTC-100TM Programmal Thermal Controller, Peltier-Effect Cycling, MJ. The amplification conditions include: initial denaturation at 94°C for 5 min; 30 cycles of a final elongation at 72°C for 5 min. For multiplex PCR of the carbapenemase genes, the annealing temperature was 55°C for amplification of *bla* $_{VIM}$, *bla* $_{IMP}$ and *bla* $_{KPC}$ genes, 45°C for *bla* $_{NDM}$ gene and 57°C for amplification of *bla* $_{OXA-48}$ genes (Karuniawati et al. 2013). The amplicons were analyzed by electrophoresis in a 1.5% agarose gel.

#### Statistical analysis

Data were statistically analyzed using Microsoft Excel 2010 and Statistical Package of Social Science (SPSS) software version 22 (SPSS Inc., Chicago, IL, USA) and categorical variables were presented as counts and percentages.

## RESULTS

### Distribution of the isolated CR Gram-negative species

During the study period, 58 CR Gram-negative species were identified from 158 GNB. The most common species were *Klebsiella pneumoniae (*37.9%), followed by *E. coli* (24.1%). Additionally, *Pseudomonas aeruginosa* accounted for 8.6%, *Proteus mirabilis* (6.9%), *Serratia marcescens* and *Enterobacter cloacae* (5.2% each), *Enterobacter aerogenes* and *Citrobacter freundii* (3.4% each), *Proteus vulgaris*, *Klebsiella oxytoca* and *Acinetobacter baumannii* (1.7% each). Most of the 58 strains were isolated from blood (63.8%), and from tracheal aspirates (20.7%). The remaining strains were isolated from umbilical venous catheter (5.2%), long line (3.4%), urine (3.4%), and others (3.4%) (Table 2).

### Antibiotic resistance pattern among CR Gram-negative isolates

The overall resistance to carbapenems was high (75.9% for imipenem and 57% for meropenem). High rate of CR was detected among *K. pneumoniae* (86.4% for imipenem and 63.6% for meropenem) and *E. coli* strains (78.6% for imipenem and 71.4% for meropenem). The best susceptibility for these highly resistant strains was detected with pipracillin/tazobactem combination (*K. pneumoniae* resistance was 18.2% and *E. coli* resistance was 14.3%). Amoxicillin/Clavulinate had some activity against few strains (only one *E. coli*, one *Enterobacter cloacae* and one *Enterobacter aerogenes* strains) (Table 3).

**Table 2.** Occurrences of CR Gram-negative species according to clinical samples.

| Species | Sample | | | | | | Total [No. (%)] |
|---|---|---|---|---|---|---|---|
| | Blood | Tracheal aspirate | UVC | Long line | Urine | Others | |
| *Klebsiella pneumoniae* | 16 | 3 | 1 | 1 | 1 | 0 | 22 (37.9) |
| *Escherichia coli* | 9 | 3 | 0 | 0 | 1 | 1 | 14 (24.1) |
| *Pseudomonas aeruginosa* | 4 | 1 | 0 | 0 | 0 | 0 | 5 (8.6) |
| *Proteus mirabilis* | 2 | 1 | 1 | 0 | 0 | 0 | 4 (6.9) |
| *Serratia marcescens* | 2 | 1 | 0 | 0 | 0 | 0 | 3 (5.2) |
| *Enterobacter cloacae* | 2 | 1 | 0 | 0 | 0 | 0 | 3 (5.2) |
| *Enterobacter aerogenes* | 1 | 0 | 1 | 0 | 0 | 0 | 2 (3.4) |
| *Citrobacter freundii* | 0 | 1 | 0 | 0 | 0 | 1 | 2 (3.4) |
| *Proteus vulgaris* | 0 | 1 | 0 | 0 | 0 | 0 | 1 (1.7) |
| *Klebsiella oxytoca* | 1 | 0 | 0 | 0 | 0 | 0 | 1 (1.7) |
| *Acinetobacter baumannii* | 0 | 0 | 0 | 1 | 0 | 0 | 1 (1.7) |
| Total No. (%) | 37 (63.8) | 12 (20.7) | 3 (5.2) | 2 (3.4) | 2 (3.4) | 2 (3.4) | 58 (100) |

UVC: Umbilical venous catheter.

**Table 3.** Antibiotic resistance rates of isolated CR Gram-negative rods.

| Species | % Antibiotic resistance rate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AMC | CXM | TZP | FOX | FEP | CRO | CAZ | CTX | IPM | MEM |
| *Klebsiella pneumoniae* (n=22) | 1 00 | 90.9 | 18.2 | 95.4 | 77.3 | 90.9 | 100 | 100 | 86.4 | 63.6 |
| *Escherichia coli* (n=14) | 92.8 | 92.8 | 14.3 | 85.7 | 71.4 | 92.8 | 92.8 | 92.8 | 78.6 | 71.4 |
| *Pseudomonas aeruginosa* (n=5) | 100 | 60 | 20 | 60 | 40 | 80 | 60 | 80 | 60 | 40 |
| *Proteus mirabilis* (n=4) | 100 | 75 | 25 | 50 | 50 | 75 | 75 | 75 | 75 | 50 |
| *Serratia marcescens* (n=3) | 100 | 100 | 0 | 66.7 | 33.3 | 100 | 66.7 | 100 | 66.7 | 33.3 |
| *Enterobacter cloacae* (n=3) | 66.7 | 66.7 | 0 | 66.7 | 33.3 | 66.7 | 66.7 | 66.7 | 66.7 | 33.3 |
| *Enterobacter aerogenes* (n=2) | 50 | 100 | 50 | 50 | 50 | 50 | 50 | 100 | 50 | 50 |
| *Citrobacter freundii* (n=2) | 100 | 100 | 50 | 100 | 50 | 50 | 100 | 100 | 50 | 50 |
| *Proteus vulgaris* (n=1) | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 0 | 100 |
| *Klebsiella oxytoca* (n=1) | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 0 |
| *Acinetobacter baumannii* (n=1) | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 0 |
| Total (n=58) | 94.8 | 87.9 | 18.9 | 82.7 | 60.3 | 86.2 | 87.9 | 93.1 | 75.9 | 57.0 |

AMC: Amoxicillin/clavulanic acid, CXM: cefuroxime, TZP: piperacillin/tazobactam, FOX: cefoxitin, FEP: cefepime, CRO: ceftriaxone, CAZ: ceftazidime, CTX: cefotaxime, IPM: imipenem, and MEM: meropenem.

### Phenotypic characterization of carbapenem resistance

ESBL production was confirmed in all 58 (100%) isolates by the cephalosporin/clavulanic acid combination disc. Detection of carbapenemase production by MHT (Figure 1) was found in 52 (89.6%) isolates while MBL production was detected in 33 (56.9%) isolates by synergy CDT (Figure 2 and Table 4).

### Genotypic characterization of carbapenem resistance

The molecular characterization of the 58 isolates showed that 57 (98.3%) were positive for carbapenemase encoding genes. *bla $_{KPC}$* was the most prevalent gene detected in 34 isolates (58.6%), next to it was the *bla $_{VIM}$* gene which was present in 15 (25.8%), *bla $_{IMP}$* in 13 (22.4%), *bla $_{OXA-48}$* in 9 (15.5%) and *bla $_{NDM-1}$* in one isolate (1.7%). Five isolates were positive for both *bla $_{OXA}$ and bla $_{KPC}$* and 4 isolates were tested positive for both *bla $_{IMP}$* and *bla VIM*. One strain was negative for all the tested genes despite positive ESBL phenotype (Table 4).

## DISCUSSION

The emergence and global spread of acquired CR isolates are designated a "global sentinel event" (Woodford et al., 2004). The overall rate of CR Gram

**Figure 1.** Modified Hodge test.

negative strains in our NICU during the study period was high (36.7%). This finding agrees with another Egyptian study conducted by Makharita et al. (2020) who detected 36% isolated Enterobacteriacea were carbapenemase producers. Much lower CR isolation rates have been reported in Turkey 2.82% (Baran and Aksu, 2016) and the United States 1.4 to 4.2% (Pollett et al., 2014). Moreover, one Chinese study reported 0.9% CR isolates (28/3286) (Liao et al., 2014). The reason for higher rate of CR detected in our study could be related to the extensive use of carbapenems to treat life threatening infections in neonatal patients and absence of antibiotic stewardship program. In this study all CR strains were retrieved from the NICU in which many CR infection and colonization factors, including impaired immune status, prolonged hospital stay, and frequent use of antibiotics, were present. For this reason, proper hand hygiene and infection control measures must be emphasized. Monitoring of CR must be encouraged to reduce CR infection rate.

*K. pneumoniae* (37.9%) was the most common CR species isolated followed by *E. coli* (24.1%). This is in

**Figure 2.** Combined disc synergy test.

agreement with other CR studies in Egypt, United States, Europe and China (Metwally and Elnagar, 2019; Centers for Disease Control and Prevention, 2013; Akova et al., 2012; Yang et al., 2018) and is consistent with the CDC's recommendations that *K. pneumoniae*, *E. coli*, and *Enterobacter* spp. are the key health care-associated pathogens to focus on in the control of US CR epidemic (Centers for Disease Control and Prevention, 2014). That is why attention should be raised when any of these bacteria is isolated from clinical specimens.

In the present study, the overall resistance to carbapenems was high (75.9% for imipenem and 57% for meropenem). Higher rate of resistance was detected by Pollett et al. (2014); Mohamed et al. (2018), who found low rate of susceptibility to meropenem (4.3%, 3%) and to imipenem (1.7%, 3%). On the other hand, lower rate of resistance was detected by Okoche et al. (2015) who found that only 18.4% of study isolates were resistant to meropenem. The varied range in susceptibility/resistance rate of carbapenems among GNB in different studies may be due to different antibiotic usage patterns in different geographic regions.

It is difficult to compare the present results with those of others taking into consideration variations in study

**Table 4.** Phenotypic and genotypic characterization of CR among 58 Gram-negative clinical isolates.

| Carbapenem resistance characterization | Number of isolates (N=58) |
|---|---|
| ***Phenotypic characterization*** | |
| **Cephalosporin/Clavulanic combination disc test:** | |
| ESBL positive | 58 |
| ESBL negative | 0 |
| ESBL (%) | 100 |
| | |
| **Modified Hodge test:** | |
| MHT-positive | 52 |
| MHT-negative | 6 |
| Carbapenemase (%) | 89.60 |
| | |
| **Synergy combined disc test:** | |
| CDT-positive | 33 |
| CDT-negative | 25 |
| MBLs (%) | 56.90 |
| | |
| ***Genotypic characterization*** | |
| $bla_{KPC}$ | 34 |
| $bla_{VIM}$ | 15 |
| $bla_{IMP}$ | 13 |
| $bla_{OXA-48}$ | 9 |
| $bla_{NDM-1}$ | 1 |
| $bla_{OXA-48}$+ $bla_{KPC}$ | 5 |
| $bla_{IMP}$ + $bla_{VIM}$ | 4 |
| PCR-negative | 1 |
| *Carbapenemase encoding genes (%)* | 98.27 |

ESBL: Extended spectrum beta-lactamase, MHT: modified Hodge test, MBL: metallo beta-lactamases, CDT: combined disc test.

populations either pediatrics or adults, different carbapenem breakpoints, and different definitions of CR (Vading et al., 2011).

In the present study, all CR strains were ESBL producers and carbapenemase production was detected in 52 isolates (89.6%), while MBL production was detected in 33 (56.9%) isolates. Similarly, Fattouh et al. (2015) identified 88.14% of CR isolates as carbapenemase producers but lower MBLs activity was detected in 33.9% of the isolates by CDT. ESBL production was confirmed by Netikul and Kiratisin (2015) in 76.2% of the CR isolates and only 14.4% were positive for MHT. In accordance, Makharita et al. (2020) detected 33.2% MBLs positive isolates while only 33.6% of the CR isolates were positive MHT. On the contrary to the present results but also in Mansoura, carbapenamase activity was detected in 61.9% of CR *K. pneumoniae* isolated from ICUs of Mansoura University hospitals by MHT method (Moemen and Masallat, 2017).

Molecular characterization of the isolates revealed that 57 (98.3%) were positive for carbapenemase encoding genes. In accordance with the present results, Makharita et al. (2020) found 98.6% (74/75) of CR strains were carrying *KPC* gene, moreover 97.3% (73/75) were carrying *GES* gene. Also, similar to the present results, Yang et al. (2018) found that, among all CR strains $bla_{KPC}$ carrying rate was 60.3% and $bla_{IMP}$ were detected in 4.5% of total CR strains. Not so far from the present results. Pollett et al. (2014) stated that, a carbapenemase-encoding gene was found in 81.7% (94/115) of CR with $bla_{KPC}$ the most prevalent (78.3%).

These results were different with Elbadawi et al. (2021) who detected that the most prevalent gene was NDM-1 gene. On the other hand, Baran and Aksu (2016). reported that the *OXA-48* gene was the most frequent gene as it was detected in 86/181 (47.5%) strains, *NDM-1* gene in 6 (3.3%) strains, and *VIM* gene in 1 (0.6%) strain. *IMP* and *KPC* genes were not identified. Both *OXA*-48 and *NDM*-1 were produced by 3 strains and both *OXA-48* and *VIM* were produced by one strain.

It is cleared that carbapenemase genes tend to be frequent in certain countries. It was found that $bla_{KPC}$

genes are dominant in USA, Greece and Egypt, while *bla* $_{NDM}$ genes are commonly detected in isolates recovered from India, Pakistan and Far East (Elbadawi et al., 2021).

A study in our locality conducted by Moemen and Masallat (2017) detected that 92.9% of the CR isolates were positive for one or more carbapenemase genes. Five of the 39 carbapenemase gene carrying isolates harbored two or more genes. The most prevalent gene was *bla* $_{KPC}$ 47.8% followed by *bla* $_{VIM1}$ 21.7%.

One strain that was negative for all the tested genes was detected despite positive ESBL phenotype. Similarly, Moemen and Masallat (2017) found that, none of the tested carbapenemase genes tested (bla $_{KPC}$, bla $_{NDM}$, bla $_{VIM-1}$, bla $_{IMP}$, and bla $_{OXA-48}$-like) were detected in three isolates. Carbapenem resistance of these isolates is mostly due to a combination of ESBLs and changes in outer membrane proteins (ESBL/Omp) (Endimiani et al., 2010).

This study had several limitations. First, the study did not check tested isolates for sensitivity to last-line antimicrobials, such as colistin, tigecycline, and fosfomycin. Secondly, only isolates that showed imipenem or meropenem in the resistant were tested. Unlikely, carbapenemases have been found in carbapenem-sensitive Enterobacteriaceae, specially *bla* $_{OXA-48}$ (Nordmann et al., 2012). Finally, clinically significant isolates were mainly examined and this undervalues the colonized patients, which may forcefully propagate CR (Viau et al., 2012). For this reason, given that CR are increasing in this region, augmentation of local infection control measures beyond core elements to active surveillance may be useful, as stressed by the CDC (Centers for Disease Control and Prevention, 2014).

## CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

### REFERENCES


Akova M, Daikos GL, Tzouvelekis L, Carmeli Y (2012). Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. Clinical Microbiology and Infection 18(5):439-448.

Al Naiemi N, Cohen Stuart J, Leverstein van Hall M (2012). NVMM Guideline Laboratory detection of highly resistant microorganisms (HRMO), version 2.0. 2012; Available: http://www.nvmm.nl/richtlijnen/hrmolaboratory-detection-highly-resistant-microorganisms

Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL (2015). European Survey of Carbapenemase-Producing Enterobacteriaceae Working Group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries. Europe's Journal on Infectious Disease Surveillance, Epidemiology, Prevention and Control 20(45).

Al-Zahrani IA (2018). Routine detection of carbapenem-resistant Gram-negative bacilli in clinical laboratories: A review of current challenges. Saudi Medical Journal 39(9):861-872.

Baran I, Aksu N (2016). Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Annals Clinical Microbiology Antimicrobials 15:20.

Centers for Disease Control and Prevention (2014). Guidance for control of carbapenem-resistant Enterobacteriaceae. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/hai/pdfs/cre/cre-guidance -508.

Centers for Disease Control and Prevention (2013). Vital signs: carbapenem-resistant Enterobacteriaceae. Morbidity and Mortality Weekly Report (MMWR) 62:165-170.

Choudhury DD, Singh NP, Rai S, Batra P, Manchanda V (2018). Carbapenem resistant Enterobacteriaceae neonatal gut colonization: A future concern in healthcare settings. Indian Journal Microbiology Research 5(3):348-354.

Clinical and Laboratory Standards Institute, CLSI (2017). Performance standards for antimicrobial susceptibility testing: Twenty-seven informational supplements; document (M100- S27). Wayne, PA: CLSI.

Elbadawi HS, Elhag KM, Mahgoub E, Altayb HN, Ntoumi F, Elton L, McHugh TD, Tembo J, Ippolito G, Osman AY, Zumla A, Abdel Hamid MM (2021). Detection and characterization of carbapenem resistant Gram-negative bacilli isolates recovered from hospitalized patients at Soba University Hospital, Sudan. BMC Microbiology 21(1):1-9.

Endimiani A, Perez F, Bajaksouzian S, Windau AR, Good CE, Choudhary Y, Hujer AM, Bethel CR, Bonomo RA, Jacobs MR (2010). Evaluation of updated interpretative criteria for categorizing Klebsiella pneumoniae with reduced carbapenem susceptibility. Journal of Clinical Microbiology 48:4417-4425.

Fattouh M, Nasr El-din A, Omar MA (2015). Detection of Klebsiella pneumoniae Carbapenemase (KPC) Producing Gram Negative Superbugs: An Emerging Cause of Multidrug-Resistant Infections in General Surgery Department of Sohag University Hospital, Egypt. International Journal of Current Microbiology and Applied Science 4(5):1-15.

Joji RM, Al-Rashed N, Saeed NK, Bindayna KM (2019). Detection of VIM and NDM-1 metallo-beta-lactamase genes in carbapenem-resistant *Pseudomonas aeruginosa* clinical strains in Bahrain. Journal of Laboratory Physicians 11(2):138-143.

Karuniawati A, Saharman YR, Lestari DC (2013). Detection of carbapenemase encoding genes in Enterobacteriacea, Pseudomonas aeruginosa and Acinetobacter baumanii isolated from patients at Intensive Care Unit Cipto Mangunkusumo Hospital in 2011. Acta Medica Indonesiana 45:101-106.

Liao K, Chen Y, Huang H, Guo P, Chen D, Liu M, Zeng Y (2014). Molecular characteristics of carbapenem-resistant Enterobacteriaceae isolates from a Chinese Tertiary hospital in Guangdong. Journal of Microbiology and Infectious Diseases 4(4):145-151.

Mahon CR, Lehman DC, Manuselis G (2007/2008). Textbook of Diagnostic Microbiology 2007. 2008. 3rd ed. St. Louis, Mo: Saunders Elsevier.

Makharita RR, El-Kholy I, Hetta HF, Abdelaziz MH, Hagagy FI, Ahmed AA, Algammal AM (2020). Antibiogram and Genetic Characterization of Carbapenem-Resistant Gram-Negative Pathogens Incriminated in Healthcare-Associated Infections. Infection and Drug Resistance 13:3991-4002.

Metwally WS, Elnagar WM (2019). Multidrug Resistant Uropathogens among Egyptian Pregnant Women. American Journal of Infectious Diseases 15(4):115-122.

Moemen D, Masallat DT (2017). Prevalence and characterization of carbapenem-resistant Klebsiella pneumoniae isolated from intensive care units of Mansoura University hospitals. Egyptian Journal of Basic and Applied Science 4(1)37-41.

Mohamed T, Yousef LM, Darweesh EI, Khalil AK, Meghezel EM (2018). Detection and Characterization of Carbapenem Resistant Enterobacteriacea in Sohag University Hospitals. Egyptian Journal of Medical Microbiology 27(4):61-69.

Mzimela BW, Nkwanyana NM, Singh R (2021). Clinical outcome of neonates with Carbapenem-resistant Enterobacteriacea infections at the King Edward VII Hospital's neonatal unit, Durban, South Africa. Southern African Journal of Infectious Diseases 36(1):a223.

Netikul T, Kiratisin P (2015). Genetic Characterization of Carbapenem-Resistant Enterobacteriaceae and the Spread of Carbapenem Resistant Klebsiella pneumonia ST340 at a University Hospital in

Thailand. PLOS ONE 10(9):e0139116.

Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V (2012). Identification and screening of carbapenemase producing Enterobacteriaceae. Clinical Microbiology and Infection Journal 18(5):432-438.

Okoche D, Asiimwe BB, Katabazi FA, Kato L, Najjuka CF (2015). Prevalence and Characterization of Carbapenem-Resistant Enterobacteriaceae Isolated from Mulago National Referral Hospital, Uganda. PLOS ONE 10(8):e0135745.

Poirel L, Walsh TR, Cuvillier V, Nordmann P (2011). Multiplex PCR for detection of acquired carbapenemase genes. Diagnostic Microbiology and Infectious Disease Journal 70(1):119-123.

Pollett S, Miller S, Hindler J, Uslan D, Carvalho M, Humphries RM (2014). Phenotypic and molecular characteristics of carbapenem-resistant Enterobacteriaceae in a health care system in Los Angeles, California, from 2011 to 2013. Journal of Clinical Microbiology 52(11):4003-4009.

Tamma PD, Simner PJ (2018). Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates. Journal of Clinical Microbiology 56(11):e01140-18.

Vading M, Samuelsen O, Haldorsen B, Sundsfjord AS, Giske CG (2011). Comparison of disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing Klebsiella pneumoniae with the EUCAST and CLSI breakpoint systems. Clinical Microbiology and Infection Journal 17(5):668-674.

Viau RA, Hujer AM, Marshall SH, Perez F, Hujer KM, Briceno DF, Dul M, Jacobs MR, Grossberg R, Toltzis P, Bonomo RA (2012). “Silent” dissemination of Klebsiella pneumoniae isolates bearing K. pneumoniae carbapenemase in a long-term care facility for children and young adults in Northeast Ohio. Clinical Infectious Disease Journal 54(9):1314-1321.

Woodford N, Tierno PM , Young K, Tysall L, Palepou MFI, Ward E, Painter RE, Suber DF, Shungu D, Silver LL, Inglima K, Kornblum J, Livermore DM (2004). Outbreak of Klebsiella pneumoniae producing a new carbapenem-hydrolyzing class A b-lactamase, KPC-3, in a New York Medical Center. Antimicrobial Agents Chemotherapy 48(12):4793-4799.

Yang Y, Chen J, Lin D, Xu X, Cheng J, Sun C (2018). Prevalence and drug resistance characteristics of carbapenem resistant Enterobacteriaceae in Hangzhou, China. Frontiers of Medicine 12(2):182-188.

Vol. 15(6), pp. 295-303, June, 2021
DOI: 10.5897/AJMR2021.9533
Article Number: 614D01C66991
ISSN: 1996-0808
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# Substitution of non-biodegradable plastic food packagings by ecological food packagings at Abomey-Calavi University (Benin): State of place and microbiological quality of packagings

**Agossou P. Noumavo[1,2*], Messan A. B. Ohin[1], Noel Tovide[1], Lucile Adjovi[1], Rachelle Dissou[1], Lamine Baba-Moussa[2], Fatiou Toukourou[1] and Farid Baba-Moussa[1]**

[1]Laboratoire de Microbiologie et de Technologie Alimentaires, Département de Biologie Végétale, Université d'Abomey-Calavi, Abomey-Calavi, Bénin.
[2]Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, Département de Biochimie et de Biologie Cellulaire, Université d'Abomey-Calavi, Abomey-Calavi, Bénin.

Received 13 April, 2021; Accepted 28 May, 2021

**Challenging with environmental problems and health due to non-biodegradable plastic wastes, Abomey-Calavi university heads have banned the use of any Non-biodegradable Plastic Food Packagings (NPFP) on all campuses. This study aimed to verify the effective implementation of this decision and assess the microbiological quality of ecological primary food packagings used in Abomey-Calavi campus. To achieve the objectives, a survey based on a questionnaire was submitted to actors in this policy (students, teachers, administrative officers and vendors). Microbiological quality evaluation of primary food packagings used consisted of counting the total mesophilic flora, total and thermo-tolerant coliforms, yeasts and molds through the basic laboratory methods. The results showed that 94,4% of Abomey-Calavi campus peoples are aware of the memo banning NPFP use within the university. They recognize that enormous risks of public health and ecosystem degradation are associated with the use of NPFP. Unfortunately, these pachagings continue to be used even though the trend is downward. The communication on ecological packagings benefits by university authorities remains insufficient. Also, some primary food packagings heavily used in this campus contains pathogenic microorganisms (thermotolerant coliforms and mycotoxinogenic molds) that can cause gastroenteritis and other food poisonings. People frequenting Abomey-Calavi campus remain permanently exposed to the risks associated with NPFP use. These packagings, especially that from food use constitutes potential and permanent sources of insalubrity, food poisoning, foodborne infection and intoxication.**

**Key words**: Plastic, packaging, microbiological quality, environmental pollution, health risk, food poisoning, foodborne infection.

## INTRODUCTION

Since prehistoric periods, packagings were used to contain a product and to preserve it from different contaminations. They also allow and make easy items transportation, distribution, storage, display, use and

marketing. Packagings are mainly made with cardboard and paper, steel and metallic materials, glass, wood and plastics (Gontard, 2015).

Today, packagings are used in almost all areas of life, including the food industry. In fact, the population growing urbanization and the development of agrifood chains in the world have increased the demand of food packagings (Contreras, 2019). This is particularly observed in developing countries like Benin Republic. It is remarked a proliferation of plastic packagings, mainly in the agri-food industries which absorb around 65% of packagings produced in the world (Benslimane, 2014). The plastic food packagings are very practical, little cumbersome, aesthetic and particularly suited to the purchasing power of low-income populations. These people live really on the income from plastic packaging activities.

Unfortunately, plastic food packagings have tremendous effects. Most of them are non-biodegradable. They become very cumbersome and create serious environmental pollution problems. Then, when used, they are obviously difficult to manage by our development countries with limited resources (Onzo et al., 2016). The management system of plastic waste is a real challenge. The West African Economic and Monetary Union reported that, the rubbish dump in Benin republic would receive more than 12,000 tonnes of plastic waste per year, more than half of which is from the packaging bags thrown into the street after use (Quenum, 2019). Better still, package hot foods ready-to-eat in plastic packagings is a daily practice embedded in african populations' habits. This african common practice creates the chemical contamination risks with sanitary harmful effects in the short or long term. Indeed, several food additives and plastic residual monomers having a negative impact on consumers' health and product quality can migrate to packaged foods, especially liquid and fatty products (Guillard and Gontard, 2017). These plastic food packagings can therefore cause chemical, physical and microbiological contaminations of foodstuffs.

Faced with this situation, the head of Abomey-Calavi University issued a memo in 2013 banning the use of any non-biodegradable plastic food packagings on all university campuses. Recently, in 2017, Beninese National Assembly examined and unanimously passed the bill n° 2017-39, prohibiting the production, importation, marketing, possession and use of non-biodegradable plastic bags in the whole country. It is in this context that this study is carried out to (i) verify the effective implementation of the university staff memo banning non-biodegradable plastic food packagings use in the Campus of Abomey-Calavi University; (ii) assess the university community knowledge level on the risks associated with the use of non-biodegradable plastic food packagings and (iii) assess the microbiological quality of ecological primary food packagings currently in use on this institution.

## MATERIALS AND METHODS

### Evaluation of the usage and risks knowledge associated with non-biodegradable food plastic packagings of university community members

The effective substitution of non-biodegradable food plastic packagings by ecological food packagings and knowledge level of university community members on the risks associated with them were evaluated through direct observations and a pre-established questionnaire. This questionnaire was filled out by students (212), teachers (26), administrative officers (22) and vendors (60). A total of 320 respondents were randomly selected at Abomey-Calavi campus in the district of Abomey-Calavi located at altitude of 12°, longitude 2° 21'20 East and latitude 6° 26'54 North in Benin Republic. It is the largest campus of Abomey-Calavi university. This list of question was relative to the sex, education level and profession of respondents; the knowledge of the ban on non-biodegradable plastic packagings use and their disadvantages, and current level use of non-biodegradable plastic packagings; the usual use of non-biodegradable plastic packagings and knowledge level of risks associated with use of non-biodegradable plastic food packagings; etc.

### Evaluation of microbiological quality of primary food packagings

Following the identification of different food packagings currently used at Abomey-Calavi campus, only main primary food packaging has been subjected to microbiological investigations.

#### *Packagings sampling*

Sixty food packagings were randomly sampled in restoration centers of Abomey-Calavi campus. Fifteen samples were collected per each type of packagings (A4 print paper, newsprint, single-use plate and multiple-use plate). Each packaging was placed in sterile sample bag and labeled. Thus, these collected samples were transported into an icebox at about 4°C to Laboratory of Microbiology and Food Technology (Abomey-Calavi university) for microbiological analysis.

#### *Packagings microbiological analysis*

The microbiological analysis consisted of stock solutions preparation and the various decimal dilutions which were subsequently inoculated on different culture media according to the microorganisms sought. Indeed, the stock solutions were prepared from the swab method. An area of 254 cm² has been delimited on

*Corresponding author. E-mail: pacome.noumavo@gmail.com.

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each packaging. This area was swabbed. Then, the swab was introduced into buffered peptone water (10 ml) and homogenized to constitute the stock solution. Decimal dilutions were prepared according to the Speck (1976) method. The mesophilic aerobic flora were grown on Plate Count Agar according to the ISO 4833:2003 standard after incubation at 30°C for 72 h. Total and fecal coliforms were isolated on Crystal Violet and Neutral Red Bile and Lactose Agar after incubation respectively at 30°C (ISO 4832: 2006 standard) and 44°C (French standard 08-060: 2009) for 24 h. Finally, the yeasts and molds were grown on Sabouraud Agar enriched with Chloramphenicol after incubation at 25°C for 3 to 5 days according to French standard NF V08-059.

#### Data processing and analysis

After manual tabulation of survey sheets, the data were encoded using Excel 2013 spreadsheet. This allowed the realization of descriptive statistics (mean, standard deviation and proportion) and various associated graphs. The data of each evaluated parameter were subjected to Analysis Of Variance (ANOVA) at probability level of 0.05 followed by the t-test "Least Significant Difference LSD" using Statistical Analysis System (SAS) version 8.1 software. Treatments were considered as a fixed factor while the repetitions were considered as a random factor (Dagnelie, 1998). Photoshop CS6 software was used to edit the different photos.

## RESULTS AND DISCUSSION

### Socio-demographic characteristics of respondents

Table 1 presents some socio-demographic characteristics of respondents involved in this study. Three hundred and twenty people including students (212), teachers (26), administrative officers (22) and vendors (60) were interviewed. This predominantly male group includes 122 women, that is, 38.13% of respondents. This gender trend is not very far from that of the university community, which between 2015 and 2016 recorded 27.8% of women (students, teachers and administrative officers) (Kpenavoun, 2017). However, this women proportion at Abomey-Calavi university (27.8%) is much lower than the national women proportion (51.2%) (INSAE, 2018). This gender difference at Abomey-Calavi university is due among other things to the low rate of girls schooling, to forced marriage, etc. The majority of respondents are literate (86.2%) and have received a university education (81.2%). This is quite normal since students constitute the majority of respondents (66.2%) and this study took place in high education institution.

### Effective substitution of non-biodegradable plastic packagings by ecological packagings

Table 2 provides information on effective substitution of non-biodegradable plastic packagings by ecological packagings at Abomey-Calavi university. About 95% of university community peoples is aware of the existence of the rector's memo banning non-biodegradable plastic packagings use within the University. Those respondents unaware of this ban mainly include newly registered high school graduates, some distance learning students and those who hardly come to classes.

This study revealed the weakness of communication on the drawbacks of the use non-biodegradable plastic packagings (NPFP) by the University authorities. In fact, 76.9% of investigated people admitted that they had hardly been posted on the dangers due to these types of packagings. But more specifically, most of vendors (66.7%) and administrative officers (90.9%) were made aware of this situation. Indeed, several awareness sessions were organized by the rectoral authorities (Hygiene and Cleaning up Service) for vendors before the ban on NPFP use. Note also that 93.4% of the surveyed students did not receive any awareness session from the rectoral authorities before the implementation of the memo banning NPFP use. A priori, one would think that students might not be associated to that the communication on the dangers of NPFP use in the campus. This is not correct. Indeed, the rectoral authorities have signed a contract concerning the drawbacks of the NPFP use with the various student union organizations. Furthermore, students were educated and well informed through several methods including general meetings, information sessions in lecture rooms and posters. Unfortunately, these student unions did not honor their commitment and the contract was broken. The university authorities should check again and find another strong alternative solution.

The study also showed that the use of non-biodegradable plastic packagings particularly for foodpurpose has decreased significantly. Most of respondents (61.2%) greatly believe that plastic packagings are less-used on the Abomey Calavi campus. These results indicate that the substitution of non-biodegradable plastic packagings by ecological packagings is not yet quite effective. According to Agossadou (2016) in the early hours of the ban of these packagings use, the whole university community was voluntarily respecting or forced to respect this interdiction because the Hygiene and Cleaning up Service of university checks were carried out every fortnight. Several vendor’s kiosks were closed during this period for not complying with this decision. Unfortunately, the control inspections were stopped. Thus, the use of non-biodegradable plastic packagings has gradually resumed on Abomey Calavi campus, especially in catering.

### Knowledge level of university community on risks associated with use of non-biodegradable plastic food packagings

The knowledge level of the respondents on the risks

**Table 1.** Socio-demographic profile of respondents

| Characteristics | Staff | Percentage |
|---|---|---|
| **Sex** | | |
| Male | 198 | 61.9 |
| Female | 122 | 38.1 |
| **Education level** | | |
| Not literate | 44 | 13.7 |
| Elementary | 16 | 05 |
| Secondary | 00 | 00 |
| University | 260 | 81.3 |
| **Profession** | | |
| Student | 212 | 66.3 |
| Administrative officer | 22 | 06.9 |
| Teacher | 26 | 08.1 |
| Vendors | 60 | 18.7 |
| **Alphabetisation** | | |
| Yes | 276 | 86.3 |
| No | 44 | 13.7 |

**Table 2.** Effective substitution of non-biodegradable plastic packagings at UAC

| Characteristics | Students (n=212)(%) | Administrativeofficers (n=22) (%) | Teachers (n=26) (%) | Vendors (n=60) (%) | Total (n=320) (%) |
|---|---|---|---|---|---|
| **Knowledge of the ban on non-biodegradable plastic packagings use** | | | | | |
| Yes | 91.5 | 100 | 100 | 100 | 94.4 |
| No | 08.5 | 00 | 00 | 00 | 05.6 |
| **Sufficient awareness of the disadvantages of non-biodegradable plastic packagings use by the university authorities** | | | | | |
| Yes | 06.6 | 90.9 | 00 | 66.7 | 23.1 |
| No | 93.4 | 09.1 | 100 | 33.3 | 76.9 |
| **Sufficient awareness of the advantages of non-biodegradable plastic packagings use by the university authorities** | | | | | |
| Yes | 05.7 | 00 | 15.4 | 20 | 08.7 |
| No | 94.3 | 100 | 84.6% | 80% | 91.3 |
| **Current level use of non-biodegradable plastic packagings** | | | | | |
| Not used | 04.7 | 00 | 00 | 10 | 05 |
| Lightly used | 64.2 | 72.7 | 69.2 | 43.3 | 61.3 |
| Moderately used | 18.9 | 09.1 | 15.4 | 46.7 | 23.1 |
| Very used | 11.3 | 18.2 | 15.4 | 00 | 10 |
| Heavily used | 0.9 | 00 | 00 | 00 | 0.6 |

associated with the use of non-biodegradable plastic packagings is summarized in Table 3. Firstly, we note that non-biodegradable plastic packagings is widely used as primary, secondary and tertiary packagings. Primary food packaging is a packaging directly in contact with the food (water bottle, can drink, etc.) while a secondary food

**Table 3.** Respondents knowledge on risks associated with use of non-biodegradable plastic food packagings.

| Characteristics | | Students (n=106) (%) | Administrativeofficers (n=11) (%) | Teachers (n=13) (%) | Vendors (n=30) (%) |
|---|---|---|---|---|---|
| **Usual use of non-biodegradable plastic packagings** | | | | | |
| Primary packaging | Food use | 66.0 | 100 | 69.2 | 80 |
| | Non-food use | 38.7 | 81.8 | 53.8 | 00 |
| Secondaire packaging | Food use | 77.3 | 72.7 | 69.2 | 16.7 |
| | Non-food use | 48.1 | 63.6 | 53.8 | 00 |
| Tertiary packaging | Food use | 38.6 | 18.2 | 53.8 | 00 |
| | Non-food use | 37.7 | 18.2 | 53.8 | 00 |
| **Knowledge level of risks associated with use of non-biodegradable plastic food packagings** | | | | | |
| Risks recognition | Yes | 98.1 | 100 | 100 | 93.3 |
| | No | 01.9 | 00 | 00 | 06.7 |
| Environmental risks | | 63.2 | 90.9 | 69.2 | 66.7 |
| Public health risks | | 79.2 | 72.7 | 76.9 | 80 |
| **Sources of informations associated with use of non-biodegradable plastic packagings** | | | | | |
| Media | | 50 | 45.4 | 38.4 | 00 |
| Academic education | | 33.9 | 27.2 | 15.4 | 00 |
| Family education | | 06.6 | 00 | 00 | 00 |
| Chat with friends | | 06.6 | 18.2 | 00 | 00 |
| Environmentalist groups/structures | | 05.7 | 18.2 | 00 | 00 |

packaging (overwrap) brings together several consumption items in a single sale unit (packs) or highlights the primary packaging (mineral water pallets). Tertiary food packaging (logistics packaging) allows several products to be transported at the same time and groups them together for transport or palletization (Nkamba, 2011).

The respondents also use these non-biodegradable plastic packagings for non-food uses. However, almost all of the intervewees recognized that there enormous public health risks are associated with use of such packagings (Table 3). It should be noted that wrapping hot ready-to-eat foods in plastic packagings is a daily practice of african populations, particularly in Benin (Figure 1C, F and G). This common practice generates risks of food chemical contamination. The effects of this contamination can be harmful in the short or long term. Indeed, several food additives and residual plastic monomers wich having a negative impact on consumer health can migrate from packaging to packaged foods, particularly for liquid and fatty products (Gontard et al., 2017).

Indeed, the bisphenol A (a major component of conventional plastic packaging) is at the center of all scientific discussions for its toxicity. Also, some new cancers would be caused or aggravated by certain particles resulting from the degradation of plastic packagings. This is the reason why it is not recommended to reuse plastic bottles (Figure 1 H). In addition, non-biodegradable plastic packagings poses environmental pollution including unhealthy cities, clogging of gutters (Onzo et al., 2013).

It is important to note that the media (radio, television, internet, etc.) and formal education constitute the main sources of information on the risks associated with the use of non- biodegradable plastic packagings in the surveyed community (Table 3). Discussions between classmates and within family units hardly address the problems related to use and management of non-biodegradable plastic packagings. In addition, the action of non-gouvernemental organizations and national institutions working in public health and environment protection is still not very perceptible even though the financial resources form national and international organizations increase every year.It is important and so urgent that african countries (particulary Benin Republic) improve their policies of food and non-food packagings management and focus communication efforts on grassroots communities, mainly in family units. Indeed, these families continue to burn non-biodegradable plastic bags for cooking fire. But how do you understand that people know and understand the risks associated with non-biodegradable plastic packagings and continue to use them?

Conventional or vegetable plastic is an exceptional

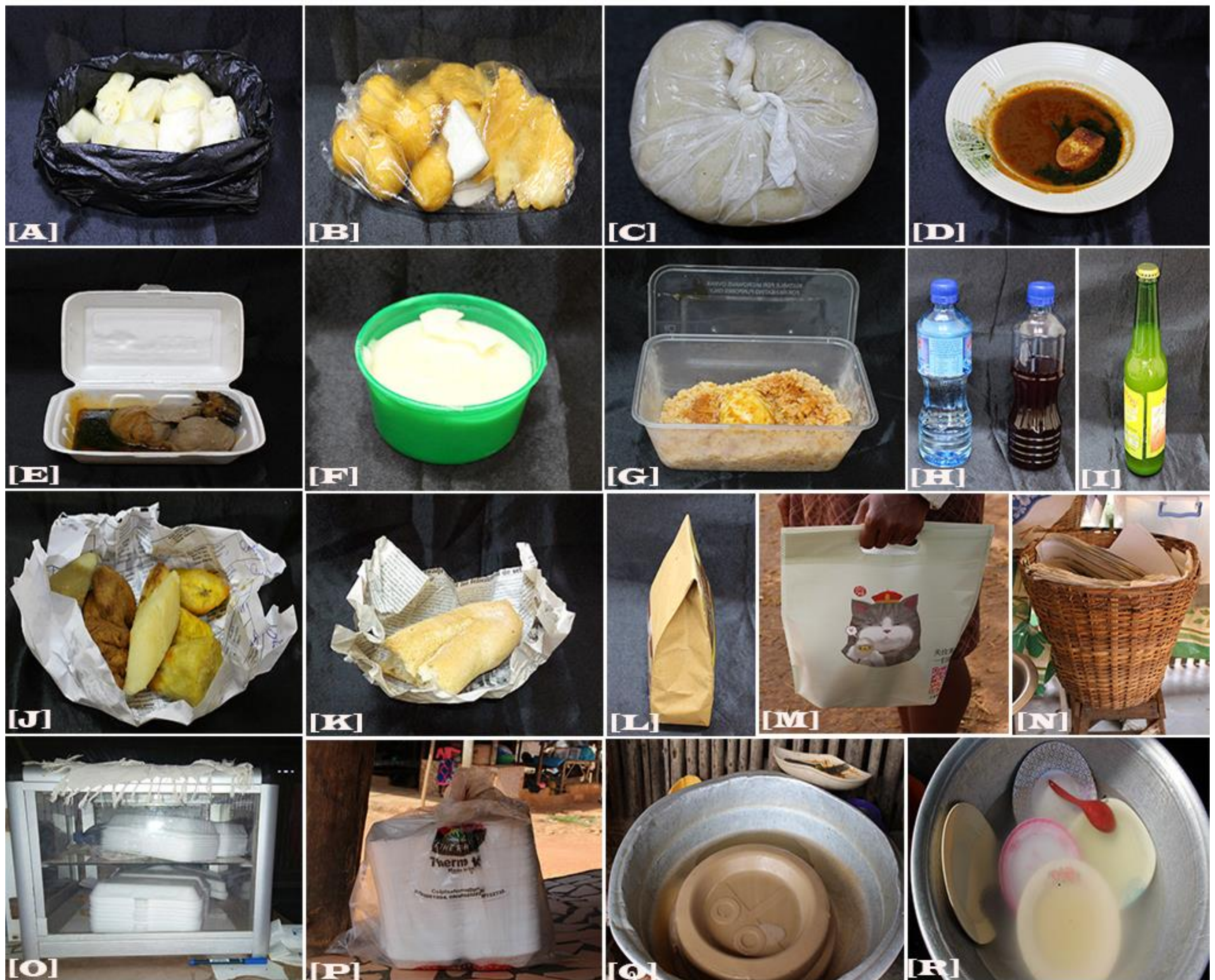


**Figure 1.** Main types of primary food packagings used on the Abomey-Calavi campus.**[A, B, C]**: Non-biodegradable plastic packagings; **[D, M]**: Non-biodegradable reusable composite packagings; **[E]**: Non-biodegradable and single-use polystyrene packagings; **[F, G, H]**: Reusable non-biodegradable plastic packagings; **[I]**: Reusable glass packaging; **[J, K, L]**: Biodegradable paper packagings; **[N, O, P]**: Some methods of packages conditioning; **[Q]**: Wash water of reusable packagings; **[R]**: Rinse water of reusable packagings.

material because of its lightness, malleability, impermeability, aesthetics, resistance, rigidity, flammability and above all its excellent quality/cost ratio (Madam, 2003). These properties are transferable to plastic food packagings. In Benin Republic, the population continues to use non-biodegradable plastic packagings because of (i) their low cost (from 0.009 $), (ii) their accessibility (available everywhere, market, kiosk, shop, etc.) (Figure 1A, B), and above all, their very low availability of biodegradable plastic food packagings or any other types of ecological food packaging.

However, these available biodegradable plastic food packagings cannot be used to package all types of food product. Indeed, the biodegradable food packaging available in Benin Republic is essentially based on paper and vegetable leaves (Figure 1J, K and L). These material cannot be used to efficiently package the large beniness food diversity. The available biodegradable plastic food packagings also tend to be more expensive than conventional plastic packaging.

In view of health and environmental damage due to the use of non-biodegradable plastic packagings and the progressive decrease in petroleum stocks, the plastics industry essentially dependent on fossil resources will

have to quickly find an alternative to the conventional raw materials it uses. One of the best alternatives is biopolymers use. Indeed, by their abundance and diversity, biopolymers offer a new source of renewable raw materials in the plastics industry. Gontard et al. (2017) reported that the main classes of vegetable biopolymers are (i) polysaccharides (starch, cellulose, agar, alginate, pectin, gums, xanthan, dextran, gellan, etc.), (ii) proteins (zein, gluten, polyamino acids, etc.), (iii) polyphenols (lignins, tannins, humic acids, etc.) and (iv) polyesters (polymers of lactic acids, polyhydroxyalkanoates, etc.). The biodegradability properties of these polymers constitute a solution to the environmental damages caused by the large tonnages of conventional plastic waste (Rabetafi ka, 2006).

**Microbiological quality of primary food packagings used on Abomey-Calavi campus**

Among the primary food packagings identified on Abomey-Calavi campus, four widely used have been subjected to microbiological analyzes. These are Pack 1, a biodegradable A4 paper packaging obtained mainly by recycling of old university thesis (PhD, Master or Bachelor) and rejects from printing works (Figure 1J); Pack 2, biodegradable newsprint packaging obtained by recycling old newspapers, unsold newspapers and rejects from printers (Figure 1 K); Pack 3, non-biodegradable single-use polystyrene packaging obtained from trade (Figure 1E) and Pack 4, non-biodegradable multi-use composite packaging obtained from commerce (Figure 1D). Since the official ban of the university authorities, Pack 1 and 2 are in heavy use. These are very accessible to vendors, almost free or at negligible costs.

The microbiological quality evaluation consisted in the enumeration of Total Mesophilic Flora (TMF), Total Coliforms (TC), Fecal Coliforms (FC) and Yeasts-Molds (YM). Analysis of variance of microbial load revealed a significant difference ($p < 0.05$) between different packagings for the parameters TC, FC and YM (Figure 2B, C and D). On the other hand, the microbial load difference of mesophilic flora is not statistically different ($p > 0.05$) (Figure 2A). There is a strong variation in the microbial load both from one type of packaging to another and within samples of the same type of packaging. The large size of standard deviations show this precedent result. This microbial load variation may be related to the application degree of good hygiene pratices which varies clearly from one vendors to another.

The paper packagings (Pack 1 and 2) did not contain coliforms (total and fecal) contrary to the packagings 3 and 4. The single-use polystyrene packaging (Pack 3) was however slightly contaminated by coliforms (Figure 1O, P). On the contrary, the coliform load was ten times more abundant in non-biodegradable multi-use packaging (Pack 4) than single-use packaging (Pack 3). This result is not surprising, because the Pack 3 was single-use and better conditioned by the vendors (Figure 1O, P) when the Pack 4 are multi-use and the dishwashing stations do not respect any catering hygienic standards. Indeed, the dishwater used by the vendors is of insufficient quality (Figure 1Q, R). Better still, it is common to see the babies or childrens of the vendors defecating or taking their baths at the sale points. These pratices contribute to the dissemination of pathogenic microorganisms from faecal origin. According to Ohin et al. (2018), the street food contamination by fecal coliforms could be linked to the use of untreated or poorly treated water, previously contaminated by animals and/or their excrement or by food handlers. Among the pathogenic germs from fecal origin, *Escherichia coli* represents 80 to 90% of fecal coliforms (thermotolerants). *E. coli* belong to natural microflora of the digestive tract of humans and warm-blooded animals (Moussé et al., 2015). But some strains are highly pathogenic. Indeed, the pathogenic microorganisms most involved in food poisoning are *Staphylococcus aureus*, *Salmonella* sp., *Clostridium perfringens* and *E. coli* (Moussé et al., 2015). *E. coli* is responsible for several diseases including the gastroenteritis. Although, it is often mild, it can sometimes have serious health consequences. Those most likely to be affected are children under the age of five, the elderly and people with weakened immune systems or with chronic diseases.

The results of this study also revealed packagings contamination by yeasts and molds (Figure 2C). The fungal load was significantly more abundant in the Packs 3 and 4 than that in the Packs 1 and 2. The most molds isolated belong to *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp. groups. The presence of these molds in primary food packagings is very worrying because they have a great toxic potential. Indeed, these molds produce several toxins named mycotoxins. Mycotoxins are among the most mutagens and carcinogens substances known. The prolonged exposure through food consumption has been linked to cancers and several diseases of the kidneys, liver and immune system (Tovide et al., 2017). It should also be noted the absence of aerobic sulfite-reducing bacteria in all investigated packagings.

The results of this study fill the informations gap on microbiological quality of food packagings both regionally and internationally area. Indeed, most of the studies carried out on microbiological quality of streed foods, both in Benin (Moussé et al., 2016; Sina et al., 2011), in Africa (Akusu et al., 2016; Bennani et al., 2016; Tchamba et al., 2015; Olorunjuwon et al., 2014; Koffi-Nevry et al., 2012; Barro et al., 2006, 2003) and worldwide (Dabboussi et al., 2013; Horton et al., 2011) focus mainly on the foodstuffs themselves and not on their packaging which is a potential and permanent source of

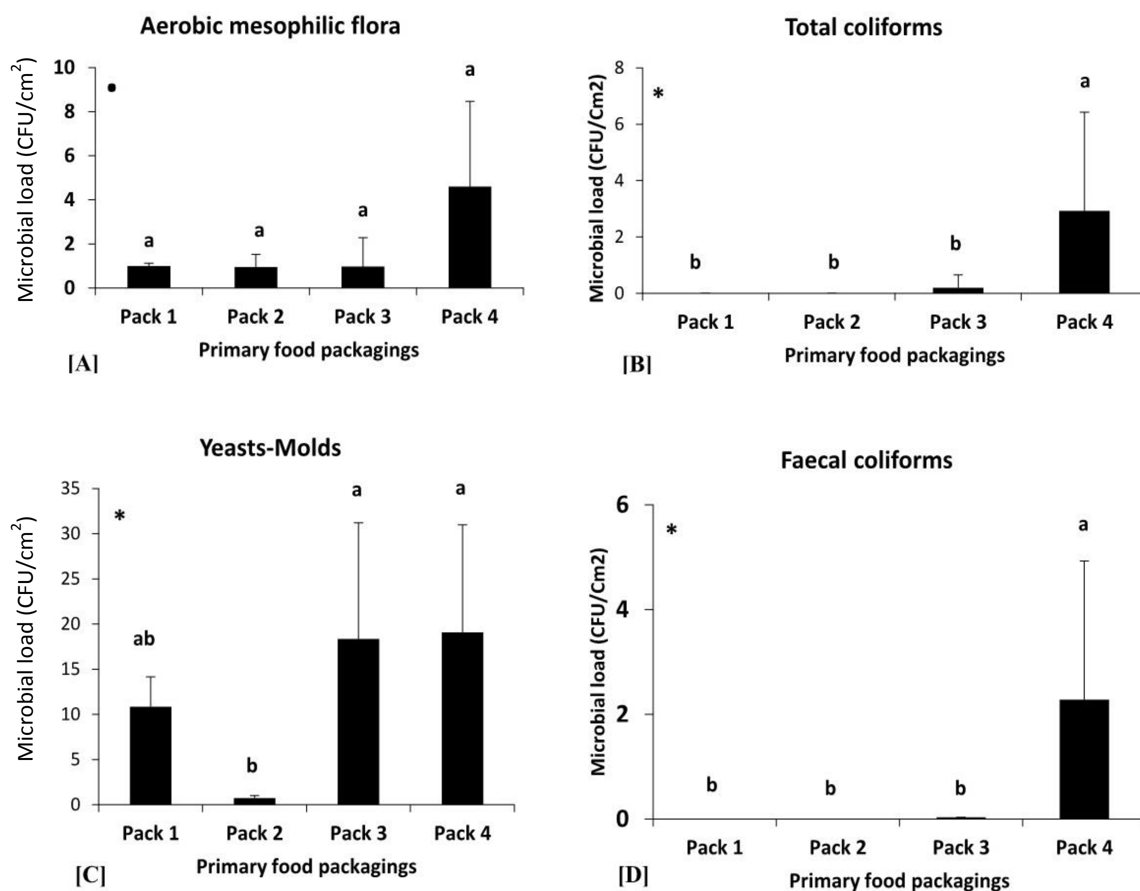


**Figure 2.** Microbiological diversity of primary food packagings used within Abomey-Calavi campus. **Pack 1** : Biodegradable A4 paper packagings ; **Pack 2** : Biodegradable newsprint packagings ; **Pack 3** : Non-biodegradable single-use polystyrene packagings; **Pack 4** : Non-biodegradable multi-use packagings. **[A] :** Distribution of aerobic mesophilic flora ; **[B] :** Distribution of total coliforms ; **[C] :** Distribution of yeasts-molds ; **[D] :** Distribution of faecal coliforms .˙ = p > 0.05 (non significant) ; * = p < 0.05 (significant) ; ** = p < 0.01 (very significant) ; *** = p < 0.001 (very highly significant). On the same graph, the means with different letters are significantly different with probability level of 5 % according to LSD (Least Significant Difference).

microbiological contamination.

## Conclusion

Almost all of the Abomey-Calavi campus people are aware of the memo banning non-biodegradable plastic packagings use within the University. They also recognize that there are more risks to public health and ecosystem degradation associated with the use of these packagings. However, these non-biodegradable plastic packagings continue to be happily used even if the trend is downward. The communication on their disadvantages and the benefits of ecological packagings by university authorities remains insufficient. This observation might be extended at the national and regional levels. Therefore, West african countries should urgently improve their policies for the management of both food and non-food packaging and concentrate communication efforts on grassroots communities, mainly family units. The adoption of ecological packagings is now a national, regional and global challenge. It should also be noticed that some primary food packagings heavily used on the Abomey-Calavi campus contains pathogenic microorganisms that can cause gastroenteritis and microbial infections of varying danger degrees. These packagings constitute potential and permanent sources of food poisoning. A public health problem therefore arises and requires the support of broth ministries of Health and Environment Protection.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## FUNDING

This study was funded by the authors.

## ACKNOWLEDGMENT

The authors thank the respondents and researchers of Laboratoy of Microbiology and Food Technology (Abomey-Calavi University) for their assistance.

### REFERENCES

Agossadou GK (2016). Conception et réalisations d'une poutre en forme de i, aux dimensions réelles et en matériaux composites tri-couche bois plastique / bois polystyrène. Mémoire de Master, Université d'Abomey-Calavi, République du Bénin P 134.

Akusu OM, Kiin-Kabari DB, Wemedo SA (2016). Microbiological quality of selected street vended foods in Port Harcourt metropolis, Rivers State, Nigeria. Sky Journal of Food Science 5(2):8-11.

Barro N, Bello AR, Savadogo A, Ouattara CAT, Ilboudo AJ, Traoré AS (2006). Hygienic status assessment of dish washing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina-Faso). African Journal of Biotechnology 5:11.

Barro N, Ouattara C, Nikiema A, Ouattara A, Traoré A (2003). Evaluation of the microbiological quality of some street foods in the city of Ouagadougou in Burkina Faso. Francophone study and research notebooks/Health 12(4):369-374.

Bennani L, Berrada S, Salame B, Aabouch M, Lalami AEO (2016). Evaluation of the hygienic quality the meat and some meat products collected from Fez city, Morocco. International Journal of Innovation and Applied Studies 15(3):547-554.

Benslimane N (2014). Contribution à l'élaboration d'un Plan de Contrôle des emballages plastiques en contact avec les denrées alimentaires. Mémoire de Master, Université de Abou Bekr Belkaïd-Tlemcen, République Algérienne Démocratique et Populaire P 58.

Contreras AG (2019). Foodyplast, food plastic packaging with naturals additives and recyclable. Université de Perpignan, France P 326.

Dabboussi F, El Omari K, Mouzawak M, Bayssari C, Hamze M (2013). Recherche de salmonella, listeria et de bactéries résistantes aux céphalosporines de troisième génération dans du fromage akkawi au nord du liban. Lebanese Science Journal 14(1):3-14.

Dagnelie P (1998). Statistique Théorique et Appliquée (vol. 1 & 2) : In De Boeck, Larcier : Presses agronomiques de Gembloux. Paris.

Gontard N (2015). L'emballage Alimentaire. L'Alimentation à découvert, Esnouf C, Fioramonti J and Laurioux B Eds, CNRS Editions, Paris, Fr. ISBN : 978-2-271-07896-4. P 90.

Gontard N, Guillard V, Gaucel S, Guillaume C (2017). L'emballage alimentaire et l'innovation écologique dans toutes leurs dimensions. Innovations Agronomiques 58:1-9.

Guillard V, Gontard N (2017). Des emballages qui ne polluent pas, ça existe! The conversation 14 février 2017.

Horton RA, Randall LP, Snary EL, Cockrem H, Lotz S, Wearing H, La Ragione RM (2011). Fecal carriage and shedding density of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli* in cattle, chickens, and pigs: implications for environmental contamination and food production. Applied and Environmental Microbiology 77(11):3715-3719.

Institut National de la Statistique et de l'Analyse Economique (INSAE) (2018). Effectif de la population béninoise, http://www.insae-bj.org/population.html, consulté le 11-05-2018 à 17 h.

Koffi-Nevry R, Assi-Clair J, Assemand E, Wognin AS, Koussemon M (2012). Origine des témoins de contamination fécale de l'eau d'arrosage de la laitue (*Lactuca sativa*) cultivée dans la zone péri urbaine d'Abidjan. Journal of Applied Biosciences 52:3669-3675.

Kpenavoun CS (2017). Quelques données statistiques de l'année académique 2015-2016. *Service Statistique*, Université d'Abomey Calavi, Bénin P 30.

Madam C (2003). La valorisation des matières plastiques en fin de vie : Etat des lieux et propositions d'amélioration. Mémoire de Master, Université Libre de Bruxelles, Belgique 95 p.

Moussé W, Sina H, Baba-Moussa F, Noumavo PA, Agbodjato NA, Adjanohoun A, Baba-Moussa L (2015). Identification of Extended-Spectrum $\beta$-Lactamases *Escherichia coli* Strains Isolated from Market Garden Products and Irrigation Water in Benin. BioMed Research International ID 286473. P 11.

Nkamba PF (2011). Influence du packaging sur l'achat des consommateurs: cas de Malta Guinness au Cameroun. Mémoire DEPA, Ecole supérieure des sciences économiques et commerciales de Douala, République du Cameroun P 76.

Ohin BM, Adéoti K, Kouhounde SS, Noumavo PA, Ogoua SM, Wabi N, Faïnou MC, Toukourou F, Baba-Moussa F (2018). Knowledge, attitudes and hygienic practices of boiled hypocotyls (*Borassus aethiopum* Mart) vended in the streets of Cotonou city and its outskirts, Benin. BioMed Research International ID 4825435. P 9.

Olorunjuwon BO, Temitope BK, Muibat FO, Oluwadun A (2014). Microbiological quality of some locally-produced fruit juices in Ogun State, South Western Nigeria. Journal of Microbiology Research 2(1):1-8.

Onzo CF, Adjatin A, Assogba F, Ndtoungou HA, Djengue HW, Azokpota P, Dansi A, Gbénou J (2016). Potentiel de domestication des espèces de feuilles végétales utilisées comme emballages alimentaires au Bénin. International Journal of Innovation and Applied Studies 18(2):539-550.

Onzo FC, Azokpota P, Akissoe N, Agbani OP (2013). Biodiversité des feuilles végétales utilisées dans l'artisanat agroalimentaire au Sud du Bénin. Journal of Applied Biosciences 72:5810-5821.

Quenum MK (2019). Plan stratégique du CREPA: 125 milliards de francs CFA pour l'hygiène, l'eau potable et l'assainissement en Afrique. IRC, https://www.ircwash.org/node/17706, 03-03-2019.

Rabetafi ka HN, Paquot M, Dubois P (2006). Les polymères issus du végétal : matériaux à propriétés spécifiques pour des applications ciblées en industrie plastique. Biotechnology, Agronomy, Society and Environment 10(3):185-196.

Sina H, Baba-Moussa F, Kayodé AAP, Noumavo PA, Sèzan A, Hounhouigan JD, Kotchoni SO, Prévost G, Baba-Moussa L (2011). Characterization of *Staphylococcus aureus* isolated from street foods: Toxin profile and prevalence of antibiotic resistance. Journal of Applied Biosciences 46:3133-3143.

Speck ML (1976). Compedium of methods for examination of food microbiological. American Public Health Associations, Washington DC pp. 417-423.

Tchamba GB, Bawa HI, Nzouankeu A, Bagré TS, Traoré AS, Barro N (2015). Isolation, characterization and antibiotic susceptibility of *Escherichia coli* and *Salmonella* spp. isolated from local beverages (bissap, gnamakoudji) sold in Ouagadougou, Burkina-Faso. International Journal of Biosciences 6(2):112-119.

Tovide N, Tchobo F, Adeoti K, Noumavo P, Baba-Moussa F, Gandonou C, Toukourou F (2017). Contamination of Cereals (*Sorghum Bicolor* L. Moench and *Pennisetum Glaucum* (L.) R. Br.) During Storage: Farmer's Perception and Management of Mold' Contamination Risks. European Scientific Journal 13(27):171-183.

Vol. 15(6), pp. 304-317, June, 2021
DOI: 10.5897/AJMR2021.9530
Article Number: D56066067000
ISSN: 1996-0808
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# Distribution patterns of Vibrionaceae abundance on the landing stages in coastal area: Understanding the influence of physicochemical variables by using multiple linear regression models and corrgram for matrix correlation

**Antoine Tamsa Arfao[1*], Mamert Fils Onana[2], Luciane Marlyse Moungang[1], Joel Brice Tchuimaleu Emadjeu[1], Blandine Pulchérie Tamatcho Kweyang[3], Olive Vivien Noah Ewoti[1], Siméon Tchakonté[1], Belengfe Sylvie Chinche[4], Télesphore Sime-Ngando[5] and Nola Moise[1]**

[1]Hydrobiology and Environment Laboratory, Faculty of Sciences, University of Yaounde 1, P. O. Box 812 Yaounde, Cameroon.
[2]Department of Aquatic Ecosystems Management, Institute of Fisheries and Aquatic Sciences, University of Douala, Douala, Cameroon.
[3]Microbiology Laboratory, University of Yaounde 1, Faculty of Sciences, P. O. Box 812 Yaounde, Cameroon.
[4]Department of Fisheries and Aquatic Resources Management, University of Buea, P. O. Box 63, Buea, Cameroon.
[5]Laboratoire Microorganismes Génome et Environnement (LMGE), Université Clermont Auvergne, Aubière Cedex, France.

Received 16 March, 2021; Accepted 13 May, 2021

**The present work used multiple linear regression (MLR) models and corrgram to assess the importance of environmental parameters on diversity and abundance dynamics of *Vibrio* sp. in waters of few landing stages in the city of Douala (Cameroon). It was recorded in all the five selected stations, the presence of four species of *Vibrio* namely, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio fluvialis* and *Vibrio alginolyticus* whose highest abundance reached 5.65, 6.26, 4.9 and 4.83 log CFU/100 ml respectively. *Vibrio cholerae* was the most isolated during the study with a frequency of 65%. The abundance dynamics of these germs is strongly influenced by nitrates, salinity, dissolved carbon dioxyde ($CO_2$) and ammonium ions ($NH_4^+$). The visualization of corrgram shows high degree of association between studied parameters. We note a coefficient of determination $r^2$ = 0.50 for the multiple linear regression model for Heterotrophic Aerobic Bacteria (HAB) and a coefficient of determination $r^2$ = 0.58 for the MLR model for *V. cholerae*. The physicochemical parameters explain at 43% ($r^2$ = 0.43) the distribution of the abundances of *V. parahaemolyticus,* at 45% ($r^2$ = 0.45) the distribution of abundances of *V. alginolyticus* and at 26% ($r^2$ = 0.26) for *V. fluvialis*.**

**Keywords**: Multiple linear regression, visualization of corrgram, environmental parameters, distribution patterns, Vibrionaceae.

## INTRODUCTION

Coastal areas are among the most important regions considering food supply and natural resources (Alizadeh

et al., 2018). They are vulnerable to the extremely variable conditions of coastal environments such as tides, storms and low flows (Conley, 2000; Gonzáles et al., 2004). This is the case of the urbanized hydrographic network of the Wouri, which is subjected to anthropogenic pressures due to the anarchic proliferation of industrial and urban activities and tidal phenomena (Tchakonté, 2016). The high activity in the coastal and marine waters can make an impact on the pollution and the quality of coastal and marine waters (Tanjung et al., 2019). Monitoring of the coastal water quality is vital from the perspectives of coastal resource usage and management (Pravakar et al., 2015). Estuaries and coastal areas are high strategic areas for economies and environment. They perform many biological and ecological functions such as fish nursery grounds. The ecological functioning of these nurseries is vital to allow the normal life cycle of many marine species of major economic interests.

Coasts are transitional surfaces between continents and the sea (Beatley et al., 1994). These are areas where the predominantly continental and oceanic mechanisms ignite and interact intensely. From an economic point of view, the coastal zone is essentially made up of consumers of fishery products and infrastructure that depend on river or sea ports (Niang et al., 2012). This structuring therefore brings out a submerged zone, made up of aquatic ecosystems (rivers, estuaries and seas) and another emerged (terrestrial) where intense economic activities take place within the limits of the coastal region which is located on either side of the coastline. Faced with multiple environmental stresses, bacteria in general and those of the genus *Vibrio* in particular have remarkable survival strategies and adaptations capacities of their physiological functions (Zhong et al., 2009).

Several studies have revealed that the dynamics of bacterioplankton abundance is generally controlled by various environmental parameters of the medium (Nola et al., 2002; Castaneda et al., 2005; Ben et al., 2014; Tamsa Arfao et al., 2021). The water quality depends of the physicochemical parameters of the waters (Hamuna et al., 2018). *Vibrio* species are known autochthonous populations found in freshwaters and marine sediments worldwide (Osunla and Okoh, 2017). However, few data are available on the distribution of vibrioplankton in Cameroonian coastal waters in general and at landing stages in particular. Little is known about the influence of physicochemical parameters on the diversity and abundance of bacteria of the *vibrio* genus in coastal areas. But, the influence of physicochemical parameters on this abundance dynamic has not been addressed very much.

Multiple linear regressions and correlation matrices are used by researchers to assess the quality of water environments. Multiple linear regression (MLR) is used to determine mathematical relationship among a number of random variables. In other terms, MLR examines how multiple independent variables are related to one dependent variable. Once each of the independent factors has been determined to predict the dependent variable, the information on the multiple variables can be used to create an accurate prediction on the level of effect they have on the outcome variable. These analysis method has been used for River water modelling prediction (Abba et al., 2017). The present work aims, through multiple linear regressions and correlation matrices, to determine the parameters which influence on the diversity and abundance of bacteria of the *Vibrio* genus at the landing stages in coastal areas.

## MATERIALS AND METHODS

### Study area and sampling stations

The study took place from February to July 2019 in the city of Douala, economic capital city of Cameroon and capital city of the Littoral Region. It is geographically located in the Golf of Guinea at the intersection of the parallel 04°03 North latitude and the 09°04 meridian East longitude. The climate is equatorial, Cameroonian type, coastal sub-type, with monomodal rainfall characterized by heavy rainfall (3414 mm on average in 2011) and an average ambient temperature of 26.3°C. There are two unevenly distributed seasons, a long rainy season which lasts nine months (March - November) and a short dry season which lasts three months (December - February) (Suchel, 1972).

Concerning the metrological data of the city of Douala during the study period, the air temperature varied from 26.1 to 27.6°C, the relative humidity as for it varies between 74 and 78%. The values of insolation and rainfall reach 190 KWh / $m^2$ / d and 592.2 mm respectively.

The vegetation, initially of the humid dense forest type, is completely degraded nowadays. The very dense hydrographic network is made up of the Wouri and Dibamba rivers and their tributaries which irrigate almost the entire city. At the petrographic level, the soils consist mainly of sandy and clayey-sandy formations (Giresse et al., 1996). In order to have a clear idea of the location of the different study sites, the geographical coordinates of all the sampling stations were determined using a Garmin Etrex 30 brand GPS. Those coordinates are given in Table 1. Figure 1 shows the geographical location of the study area and the sampling stations.

The choice of sampling stations was made on the basis of their accessibility, their economic importance and their proximity to marketplaces. These different stations serve as clearinghouse for the landing of sea products of various species (shrimp, fish, skate), and the risks of contamination of the landing stages are higher. A total of five stations were chosen including two stations at the Youpwe landing stage (Youpwe 1 and 2), one station at the Essengue and Akwa North landing stages and a last station at the Sandaga port. At each site, water sample was collected in a 500 ml

*Corresponding author. E-mail: tamsarfao@yahoo.fr.

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**Table 1.** Geographic coordinate of the sampling stations.

| Geographic coordinate | Sampling stations | | | | |
|---|---|---|---|---|---|
| | Youpwe 1 | Youpwe 2 | Akwa North | Essengue | Sandaga |
| Altitude (m) | 0 | 0 | 11 | 3.6 | 25 |
| Latitude | 04°01`26,7`` | 04°01`35 `` | 04°04`58`` | 04°2`267`` | 04°03`43,3`` |
| Longitude | 09°40`0,6`` | 09°40`02`` | 09°42`41`` | 09°40`06`` | 09°41`57,7`` |

**Figure 1.** Map of the study area showing sampling stations

sterile glass bottle labeled A, and in a 1000 ml clean polyethylene bottle labeled B. Both samples were transported to the laboratory in a cooler with icepacks (7±2°C) for further analyses. The sample in bottle **A** and that in the polyethylene bottle **B** were for the assessment of the bacterioplankton cells and for some physico-chemical analysis respectively.

**Measurement of environmental variables**

At the level of each sampling station, the physicochemical analysis focused on 12 variables. Physicochemical parameters (Water Temperature (°C), pH (CU), dissolved oxygen (% of saturation), electrical conductivity of water (µS / cm), salinity (psu), dissolved $CO_2$ (mg/L), suspended Solids (mg/L), water color (Pt.Co), turbidity (FTU), nitrates (mg/L), orthophosphates (mg/L of $PO_4^{3-}$) and ammonium ions (mg/L of $NH_4^+$)) were measured according to the techniques described by APHA (2009) and Rodier (2009).

**Bacterial isolation and identification**

The quantitative analysis of bacteria aimed at isolating and counting of heterotrophic aerobe bacteria (HAB) and bacteria of the genera *Vibrio* sp. The analysis technique used was that of surface spreading on Agar culture media poured into Petri dishes. HABs were isolated on ordinary agar medium, incubated at 22°C for 5 days (Tamsa Arfao., 2021). Thiosulfate Citrate Bile Salts (TCBS ; BioMerieux) was used for the isolation *Vibrio* sp, incubated at the temperature of 37°C for 24 h. The Identification of *Vibrio* species

began with the research for mobility type and Gram staining. Gram stain provides information on the morphology and structure of the bacteria wall. The parameters considered were the colour, size and shape of the colonies on the TCBS agar. Classical biochemical identification has made it possible to search for enzymes such as oxidase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophanase and βgalactosidase (Holt et al., 2000). From a pure culture colony on alkaline nutrient agar, a suspension in sterile distilled water was prepared. The bacterial suspension were distributed in the capsules of the API 20E system (BioMérieux, France). The metabolites produced were made evident by color reactions or by addition of reagents, after 24 h of incubation at 37°C. Corresponding bacterial species were determined numerically with the help of APIDENT 2.0 software. The identification rate was maintained at least at 98%. The count of isolated germs was carried out using an OSI brand colony counter. Bacterial abundances are expressed in decimal logarithmic units Colonial Forming Units (CFU) per 100 ml of water sample.

### Data and statistical analysis

Data was typed using Microsoft Excel and imported into the programme SPSS version 25.0 for analysis. The Kolmogorov-Smirnov test was first applied to check the normality of the distribution before comparing environmental parameters and abundances of bacteria isolated. The Kruskal – Wallis test and Mann – Whitney test were then performed with SPSS 25.0 to verify significant differences between stations considering each environmental parameter. The corrplot function of R Software version 3.5.0 allowed us to obtain corrgram. A corrgram represents the graph of a correlation matrix. The corrgram is very important to highlight the most correlated variables. In this type of graph, the correlation coefficients are colored according to their value. The correlation matrix can also be reordered according to the degree of correlation between the variables. This corrgram was used to measure the degree of association between abiotic variables on one hand, and between abiotic and biological variables on the other hand. Multiple linear regression models were done to establish relationships between the physicochemical variables and abundance bacteria of *Vibrionacaea*. This statistacal method predicts the level of variation between the variables and defined by the following equation:

$$y_i = b_0 + b_1 x_1 + b_2 x_2 + \cdots + b_i x_i$$

Where, for i = n observations, $y_i$ is dependent variable, $x_i$ is explanatory variables, $b_0$ is y-intercept (constant term) and $b_i$ the slope coefficients for each explanatory variable (Parmar and Bhardwaj, 2015; Chen and Liu, 2015).

## RESULTS

### Environmental variables

The minimum and maximum values, the mean values and standard deviations of the physicochemical parameters measured at each landing stage studied are presented in Table 2. The results of the comparison tests of the Kruskal Wallis test have also been presented. The water temperature and pH values varied from 28 to 31.44°C and from 7.81 ± 1.02 and 9.14 ± 0.99 CU. The average temperature values fluctuated between 29.03 ± 1.33 and 30.57 ± 0.91°C with a high value recorded in March at the Essengue landing stage. The lowest pH value was recorded in February at Youpwe 2 and the highest value in June at Akwa North landing stage. Dissolved $CO_2$ content varied with mean values between 52.89 ± 12.80 and 91.06 ± 63.62 mg/L. No significant difference was observed between the stations (Kruskal-Wallis H test; $p > 0.05$) for these two parameters. The same was true for the pH values, which also showed no significant difference between the different landing stages.

Suspended solids contents were relatively high at most stations throughout the sampling period. The mean values ranged from 14.33 ± 3.14 mg/L to 49.50 ± 30.7 mg/L. The evolution of suspended solids content showed significant differences (Kruskal-Wallis H test; $p < 0.05$), in particular between the Youpwe 1 and Sandaga wharves, between Youpwe 2 and Akwa North, and between Essengue and Akwa North (Mann-Whitney U test; $p < 0.05$). Turbidity contents ranged from 10 to 153 NTU. The lowest values were obtained at the Sandaga stations and the highest in March at the Akwa North station. Water color fluctuated between 17 and 447 Pt.Co during the study. The variation profile of these two parameters does not show any significant difference between the different stations studied ($p > 0.05$). Regarding ammonium ions, significant differences ($p < 0.05$) were observed with mean values between 0.13 ± 0.08 and 0.49 ± 0.27 mg/L. The differences observed were between Youpwe 1 and the other landing stages except those of Youpwe 2 and Akwa North ($p < 0.05$). Water salinity showed relatively stable spatial fluctuations during the study period. However, the highest salinity concentration was obtained in March in Essengue (12.28 psu) and the lowest in July at the Akwa North landing stage (0.29 psu) with average values between 0.38 ± 0.07 and 10.05 ± 1.58 psu. Variations in salinity were significant between all the stations (Kruskal-Wallis H test and Mann-Whitney U test; $p < 0.05$), except between the Youpwe 1 and Essengue docks where there is no significant difference ($p > 0.05$). Dissolved oxygen saturation rate had mean values between 60.93 ± 9.65 and 70.85 ± 8.37%, the greatest value being observed at the Sandaga landing stage. The same statistical results are observed for waters which content nitrates and phosphates (Kruskal-Wallis H test; $p > 0.05$). Electrical conductivity varied between 10.1 and 767 µS/cm at the landing stages of Essengue and Akwa North respectively. A significant difference was recorded ($p < 0.05$), in particular between the Youpwe 1 and Akwa North wharves, between Youpwe 2 and Akwa North. The lowest nitrate content in the water was obtained in Sandaga in April (1.8 mg/L) while the highest nitrate concentration was 13.22 mg/L. This value was recorded in Essengue in June. The highest level of phosphates reached the value of 1.591 mg/L, observed at the Youpwe 1.

### Biochemical characterization of isolates

The cultural characters of the different *Vibrio* species

**Table 2.** Physicochimie metric for different stations studied.

| Metric | Stations | | | | | K-W test |
|---|---|---|---|---|---|---|
| | Youpwe 1 | Youpwe 2 | Akwa North | Essengue | Sandaga | |
| Temperature (°C) | 28 - 30.5<br>29.58 ± 0.92 | 29 - 31<br>30.25 ± 0.76 | 29 - 31<br>30.26 ± 0.99 | 29 - 31.44<br>30.57 ± 0.91 | 28 - 31.2<br>29.03 ± 1.33 | H = 6.944<br>P = 0.139 |
| Dissolved $CO_2$ (mg/L) | 17.5 - 167.2<br>68.35 ± 70.24 | 44 - 190.8<br>91.06 ± 63.62 | 34.44 - 68.3<br>52.89 ± 12.80 | 21.28 - 190.1<br>57.49 ± 65.37 | 32.08 - 93.56<br>67.51 ± 23.07 | H = 5.454<br>P = 0.244 |
| pH (C.U) | 6.2 - 9.3<br>7.94 ± 1.21 | 4.82 - 9.2<br>7.87 ± 1.68 | 7.4 - 10.7<br>9.05 ± 1.51 | 6.47 - 9.04<br>7.81 ± 1.02 | 7.37 - 10.09<br>9.14 ± 0.99 | H = 6.190<br>P = 0.185 |
| Salinity (psu) | 7.02 - 9.59<br>$8.67 \pm 0.88^{a}$ | 6.98 - 8.01<br>$7.41 \pm 0.45^{b}$ | 0.29 - 0.47<br>$0.38 \pm 0.07^{c}$ | 8.2 - 12.28<br>$10.05 \pm 1.58^{d,a}$ | 1.6 - 2.13<br>$1.96 \pm 0.21^{e}$ | H = 25.948<br>P = 0.000 |
| Dissolved oxygen (%) | 45 - 74.4<br>63.4 ± 11.01 | 49 - 77.1<br>60.93 ± 9.65 | 46.1 - 71<br>61.80 ± 9.61 | 67.1 - 73<br>69.58 ± 2.55 | 55.8 - 79.1<br>70.85 ± 8.37 | H = 5.929<br>P = 0.205 |
| Nitrates (mg/L $NO^{3-}$) | 2 - 12.1<br>$8.37 \pm 4.49^{a}$ | 3.8 - 10<br>7.03 ± 2.84 | 2.1 - 12<br>7.5 ± 3.29 | 6.2 - 13.22<br>10.54 ± 2.63 | 1.8- 11.23<br>3.17 ± 3.14 | H = 5.786<br>P = 0.216 |
| Suspended solids (mg/L) | 9 - 33<br>$17.33 \pm 8.31^{a}$ | 5 - 32<br>$23.50 \pm 9.75^{b,a}$ | 13 - 100<br>$49.50 \pm 30.7^{c,a}$ | 11 - 20<br>$14.33 \pm 3.14^{d,b,a}$ | 18 - 70<br>$40.17 \pm 22.43^{e,b,c,d}$ | H = 12.815<br>P = 0.012 |
| Conductivity (µS/cm) | 13.28 - 16.8<br>$15.13 \pm 1.32^{a,e}$ | 13.21 - 166<br>$58.2 \pm 68.89^{b,a}$ | 137 - 767<br>$336.83 \pm 300^{c}$ | 10.1 - 194<br>$64.42 \pm 84.48^{d,a,b,c}$ | 11.12 - 384<br>$107.1 \pm 144.8^{e,b,c,d}$ | H = 10.135<br>P = 0.038 |
| Tubidity (NTU) | 53 - 104<br>78.67 ± 20.57 | 38 - 78<br>66.17 ± 17.01 | 28 - 153<br>85.17 ± 57.24 | 21 - 58<br>41.17 ±13.41 | 10 - 147<br>56.50 ± 63.47 | H = 6.709<br>P = 0.152 |
| Color (Pt-Co) | 17 - 179<br>101.5 ± 66.64 | 36 - 149<br>63.67 ± 42.79 | 26 - 447<br>204 ± 200.2 | 30 - 99<br>61.67 ± 32.86 | 100 - 355<br>163.67 ± 97.0 | H = 5.393<br>P = 0.249 |
| Phosphates (mg/L $P0_4^{3-}$) | 0.22 - 1.59<br>0.66 ± 0.51 | 0.14 - 1.56<br>0.67 ± 0.65 | 0.08 - 1.32<br>0.52 ± 0.46 | 0.12 - 0.9<br>0.34 ± 0.29 | 0.21 - 0.86<br>0.42 ± 0.23 | H = 2.034<br>P = 0.729 |
| Ammoniacal nitrogen (mg/L $NH_4^{+}$) | 0.09 - 0.76<br>$0.43 \pm 0.24^{a}$ | 0.06 - 0.75<br>$0.49 \pm 0.27^{b,a}$ | 0.1 - 0.49<br>$0.32 \pm 0.14^{c,a,b}$ | 0.03 - 0.22<br>$0.13 \pm 0.08^{d}$ | 0.06 - 0.17<br>$0.13 \pm 0.04^{e,d}$ | H = 12.502<br>P = 0.014 |

Values represent Min – Max & mean ± standard deviation. Kruskal–Wallis (K–W) tests were used to evaluate differences among the four groups. In the same row, values followed by different superscripts (a,b,c,d,e) are significantly different (Kruskal–Wallis test).

listed were yellow and flat colonies of 2 to 3 mm in diameter, presumptive of *Vibrio cholerae*, then yellow colonies of large size presumptive of *Vibrio alginolyticus*, then yellow or translucent colonies presumptive of *Vibrio fluvialis* and *Vibrio vulnificus* and finally those which were colorless with green center, presumptive of *Vibrio parahaemolyticus*.

The biochemical tests carried out using the API20E system from the colonies isolated on TCBS made it possible to have the biochemical profile of the strains studied as presented in the Table 3. Overall, it emerges that all the isolated species are positive in the tests for oxidase, glucose, mannitol and nitrates. They are all negative on urease and lactose tests and do not produce gas.

However, only *V. parahaemolyticus* is negative to the sucrose test while the others (*V. alginolyticus, V. cholerae and V. fluvialis*) are positive. *V. fluvialis* species are distinguished by their positivity in the ADH (arginine di-hydrolase)

**Table 3.** Identification tests carried out and different species isolated.

| Biochemical tests | Bacteria species | | | |
|---|---|---|---|---|
| | *V. parahaemolyticus* | *V. fluavilis* | *V. cholarae* | *V. alginoticus* |
| Oxydase | + | + | + | + |
| Urease | - | - | - | - |
| Glucose | + | + | + | + |
| Lactose | - | - | - | - |
| Gas | - | - | - | - |
| $H_2S$ | - | - | - | - |
| Mannitol | + | + | + | + |
| Mobility | + | + | + | + |
| Saccharose | - | + | + | + |
| Nitrates | + | + | + | + |
| ONPG | - | + | + | - |
| Indole | + | - | + | + |
| Gelatinase | + | + | + | + |
| Citrate | - | + | + | - |
| LCD | + | - | + | + |
| ODC | + | - | + | +/- |
| ADH | - | + | - | - |
| Colony color | Green | | Yellow | |

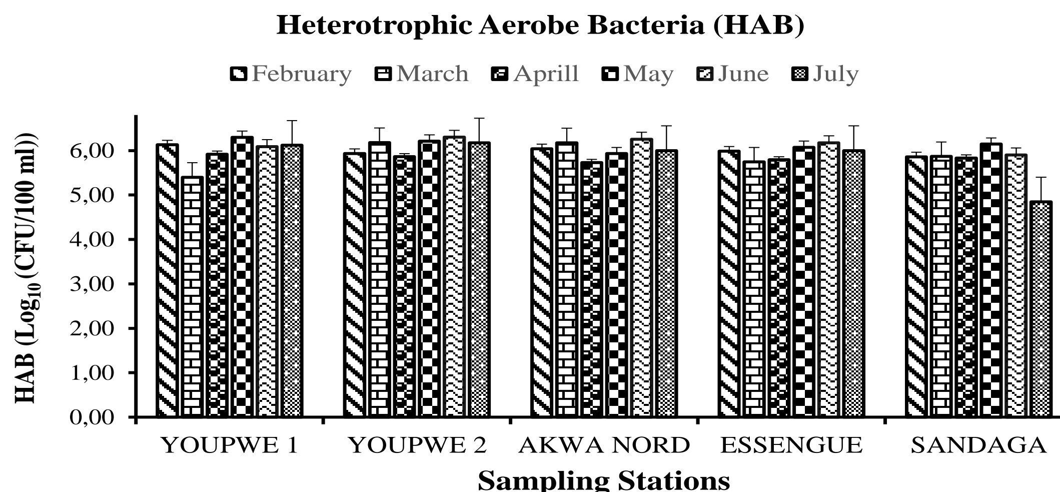


**Figure 2.** Spatio-temporal variations in cell abundance of Herotrophic Aerobe Bacteria.

test, while all the others are negative in this test. Finally, *V. alginolyticus* and *V. cholerae* are distinguished by their reactivity or not to citrate. Citrate is degraded by *V. cholerae* but not by *V. alginolyticus* because it does not use it as a source of carbon for its food needs.

## Spatiotemporal variations of studies bacteria

The spatiotemporal variations in the abundance of bacterial germs are presented in Figures 2 and 3. These are HAB and *V. cholerae*, *V. alginolyticus*, *V. fluvialis* and *V. parahaemolyticus*. The abundances of HAB cells, expressed in decimal logarithmic units (CFU/100 ml), ranged from 4.73 to 6.32. The lowest value was observed in March at Akwa North wharf and the highest abundance at Youpwe 2 wharves in April and July respectively (Figure 2). Cell concentrations of *V. cholerae* and *V. parahaemolyticus* fluctuated from 3.30 Log10 units (CFU/100 ml) to 6.26 Log10 units (CFU/100 ml) and from 0 to 5.6 Log10 units (CFU/100 ml), respectively (Figure 3A and B). The abundances of *V. alginolyticus* were

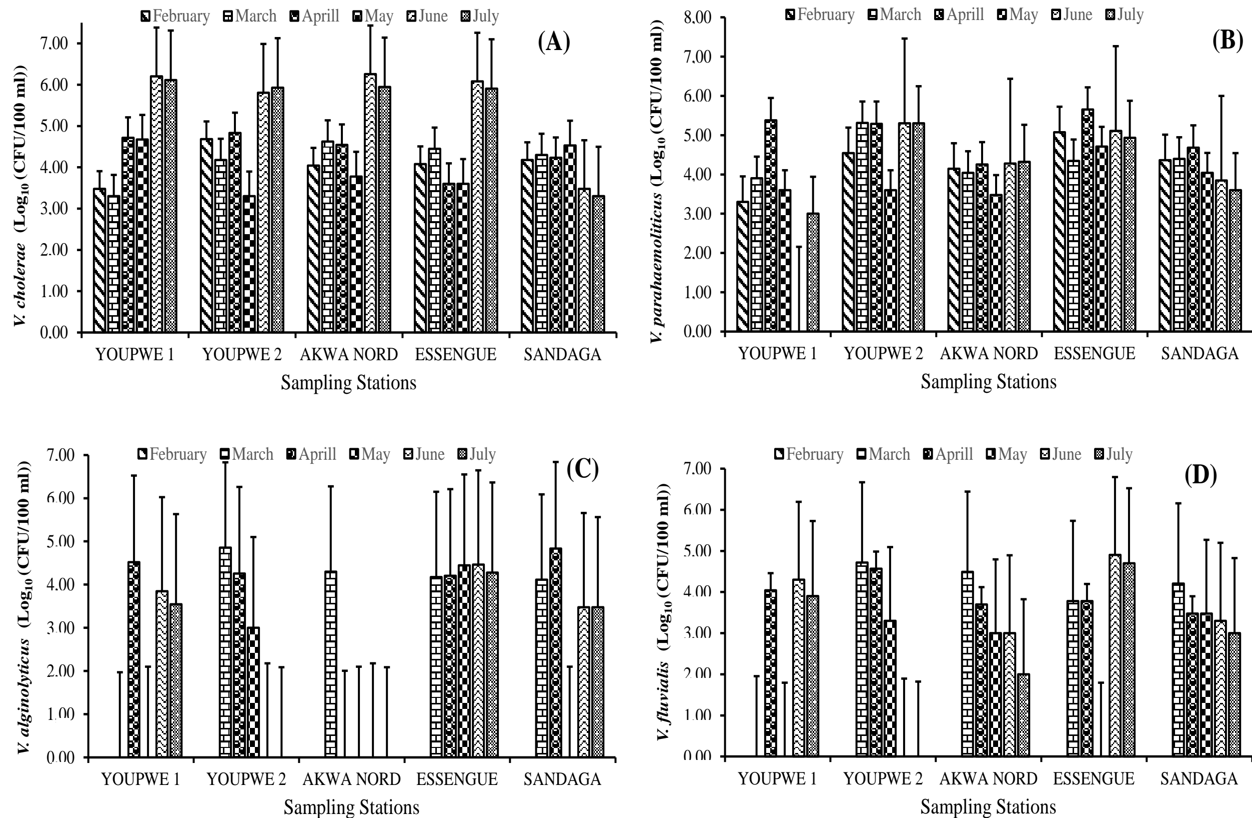


**Figure 3.** Spatio-temporal variations in cell abundance of *Vibrio cholerae* (**A**), *Vibrio parahaemolyticus* (**B**), *Vibrio alginolyticus* (**C**), *Vibrio fluvialis*.

between 0 and 4.86 Log10 units (CFU/100 ml) (Figure 3D). The densities of *V. fluvialis* ranged from 0 to 4.90 Log10 units (CFU/100 ml) (Figure 3D).

## Relative frequency of bacteria isolated

Figures 4 and 5 shows the relative frequencies of bacteria isolated at each study station. In Youpwe 1 station, HAB predominated with a relative frequency of 92%. *V. cholerae* was the second most abundant group (6%). The relative frequencies of the other organisms were 1% for *V. parahaemolyticus*, 1% for *V. fluvialis* and 0% for *V. alginolyticus*. In station Youpwe 2, HAB dominated with a relative frequency of 76% followed by *V. cholerae* (15%), then *V. parahaemolyticus* (7%), then *V. fluvialis* and *V. alginolyticus* with 1% respectively. At Akwa North station the relative frequencies are 70% for HAB, 29% for *V. cholerae*, 1% for *V. parahaemolyticus*, 0% for *V. fluvialis* and *V. alginolyticus* respectively. In the Essengue station, HAB predominated with a relative frequency of 65%. *V. cholerae* was the second most abundant group (23%), the relative frequencies of the other organisms were 9% for *V. parahaemolyticus*, 2% for *V. fluvialis* and 1% for *V. alginolyticus* (Figure 4). In the Sandaga station, HAB dominated with a relative frequency of 93% followed by *V. cholerae*, *V. parahaemolyticus* and *V. alginolyticus* with 2% respectively. *V. fluvialis* is the last group with a relative frequency of 1% (Figure 5).

## Corrgram of studied parameters

Each correlation in the correlation matrix is represented by a disk which color and size are directly related to the correlation represented. The corrgram obtained from correlation matrix is presented on Figures 6 to 8. In the Youpwe 1 station, the most salient significant correlations were recorded between HAB and dissolved $CO_2$, suspended solids and orthophosphates (negative correlations) on one hand, and with temperature nitrates and ammonium ions (positive correlations) on the other hand. *V. chlorae* is positively and significantly correlated with pH and nitrates, and negatively correlated with turbidity. Ammonium ions and orthophosphates, on their part, positively influenced the abundances of *V. parahaemolyticus* and *V. alginolyticus* while there is a significant and negative correlation between these two species and dissolved oxygen (Figure 6A). In Youpwe 2 station, pH, nitrates and suspended solids significantly and positively influenced the distribution of HAB and

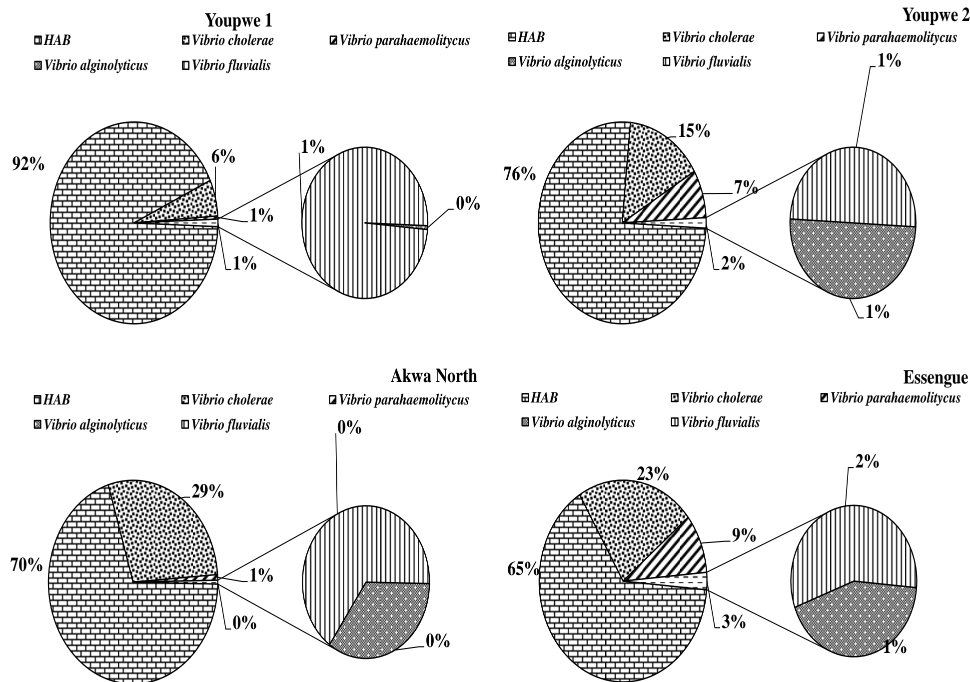


**Figure 4.** Relative frequency of each group of bacteria studied at the landing stages of Youwpe 1, Youwpe 2, Akwa North and Essengue.

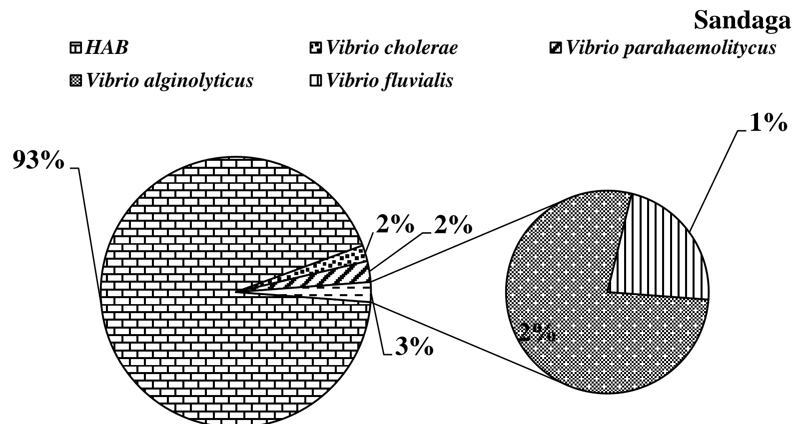


**Figure 5.** Relative frequency of each group of bacteria studied at the landing stage of Sandaga.

*Vibrio cholerae* while salinity and dossolved $CO_2$ had a negative influence (Figure 6B). At the AKWA landing stage, there is a significant and negative correlation between HAB and Ammonium ions while temperature positively influences their distribution. *Vibrio cholerae* is positively influenced by pH, temperature and dissolved oxygen. Salinity significantly and negatively influences the distribution of *V. cholerae* and *V. parahaemolyticus*. Nitrates, electrical conductivity, turbidity and colour positively influenced the distribution of *V. alginolyticus* and V. *fluvialis* while ammonium ions and pH negatively influenced their distribution in this station (Figure 7A).

At the Essengue station, the most prominent correlations are observed between temperature, salinity, electrical conductivity, turbidity, colour, HAB, *Vibrio cholerae*, *V. alginolyticus* and *V. fluvialis* (significant and negative correlations). On the other hand, positive correlations are observed with pH and nitrates (Figure 7B). At the Sandaga station, a significant and positive correlation was noted between HAB, *V. cholerae* and salinity. No negative correlation was noted between HAB and the physicochemical parameters at this station.

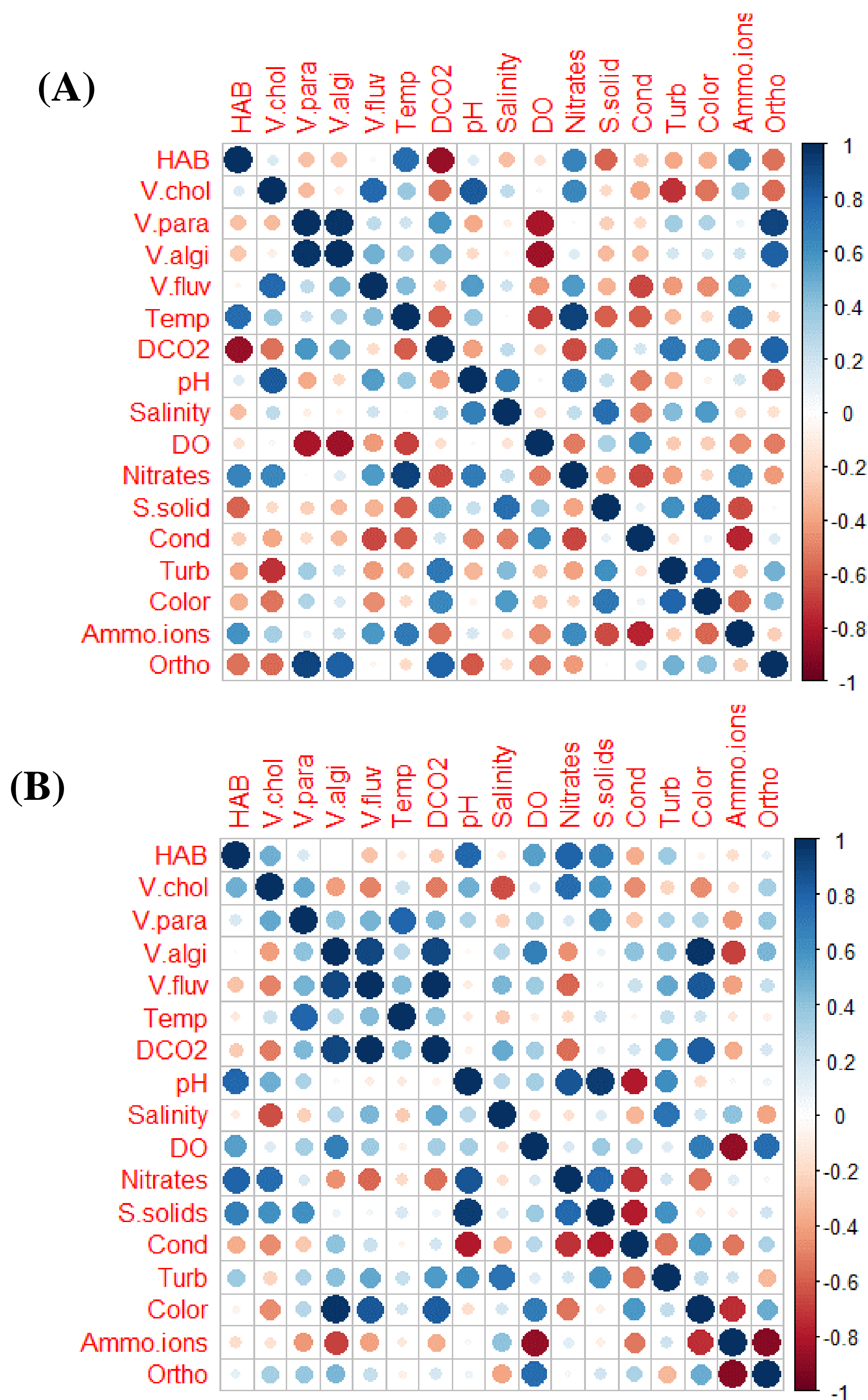


**Figure 6.** Corrgram for studied parameters at the landing stages of Youpwe 1 (**A**) and Youpwe 2 (**B**), correlations shown by color and intensity of shading.

Strong significant and negative correlations are noted between *V. parahaemolyticus*, *V. alginolyticus,* pH and nitrates. *V. fluvialis* is influenced significantly and negatively by dissolved oxygen and Ammonium ions and significantly and positively correlated with temperature, dissolved $CO_2$, suspended solids, electrical conductivity,

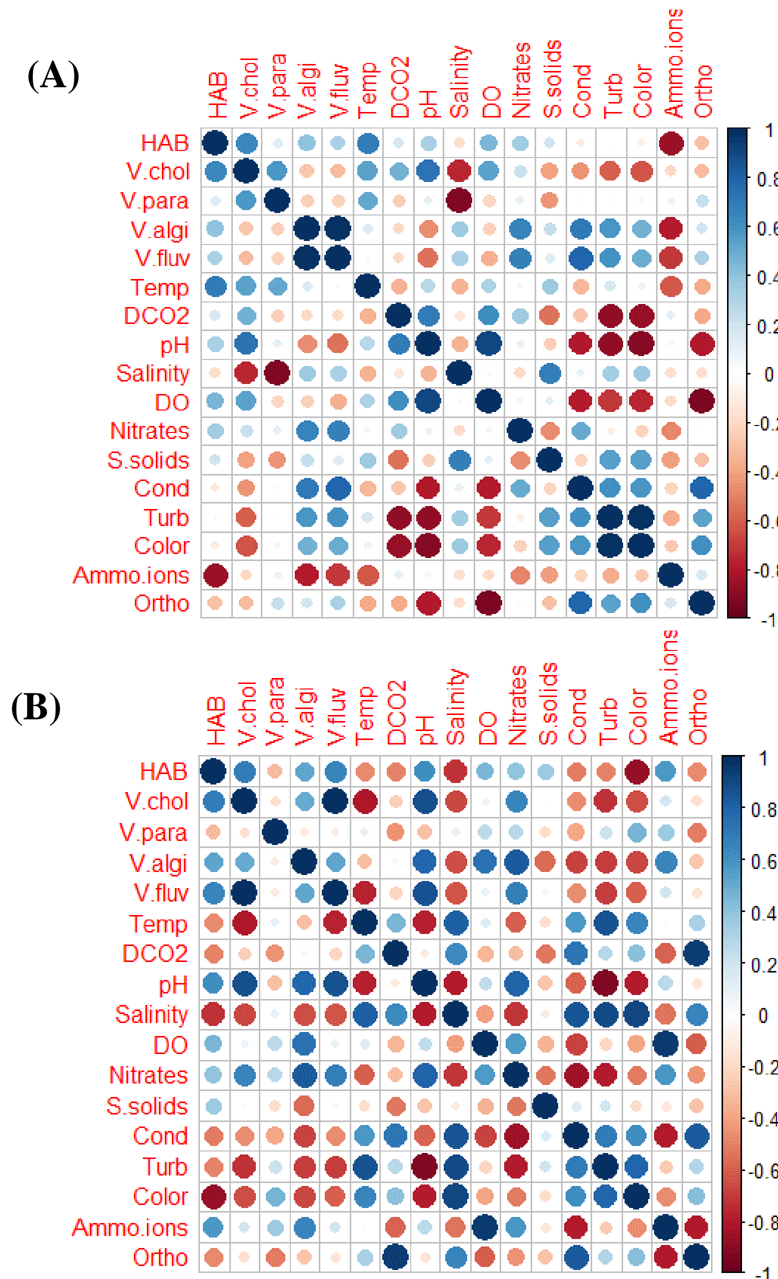


**Figure 7.** Corrgram for studied parameters at the landing stages of Akwa Nord (**A**) and Essengue (**B**), correlations shown by color and intensity of shading.

turbidity, color and orthophosphates (Figure 8).

## Multiple linear regression analysis between bacterial abundance and abiotic factors

The analysis of the multiple regressions between the abundances of isolated bacteria and the physicochemical variables in the different stations is presented in Table 4.

The model equations explaining the distribution of bacteria have made it possible to observe some important relationships based on the coefficient of determination ($r^2$) which measures the accuracy of the prediction of the distribution of the bacterial abundances studied. We therefore note a coefficient of determination $r^2 = 0.50$ for the multiple linear regression model for HAB and a coefficient of determination $r^2 = 0.58$ for the multiple linear regression model for *V. cholerae*. Furthermore, for the bacteria *V. parahaemolyticus, V. alginolyticus* and *V. fluvialis*, the model explains less than

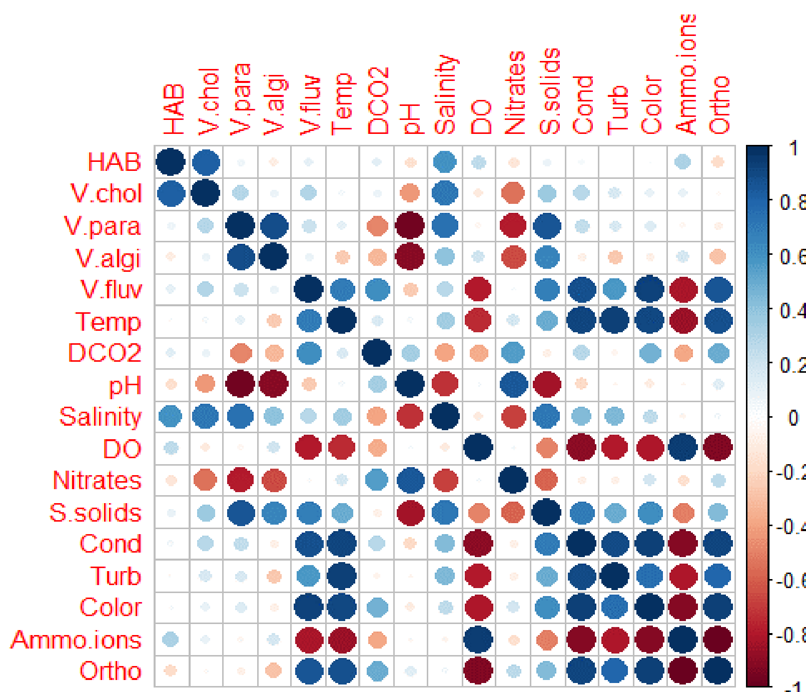


**Figure 8.** Corrgram for studied parameters at the landing stage of Sandaga, correlations shown by color and intensity of shading.

**Table 4.** Results of Multiple linear regression models between the physicochemical variables and abundance bacteria.

| Dependent variables | n | Model equation | F | $r^2$ |
|---|---|---|---|---|
| Heterotrophic aerobe bacteria | 30 | $- 54850.17 + 1149.39*Temp - 16.42*DCO^2 - 288.52*pH - 18.53*Sali + 328.07*DO + 586.98*NO_3^- + 8130*SS + 2.10*Cond + 20.92*Turb - 11.62*Col + 13971.30*NH_4^+ + 3713.94*PO_4^{3-}$ | 1.39 | 0.50 |
| *V.cholerae* | 30 | $- 60747.20 + 1363.89*Temp - 46.58*DCO^2 + 3239.60*pH + 679.89*Sali - 43.62*DO - 307.90*NO_3^- + 22.62*SS + 8.65*Cond - 44.62*Turb - 9.64*Col + 2984.76*NH_4^+ + 3967.77*PO_4^{3-}$ | 1.95 | 0.58 |
| *V. parahaemolyticus* | 30 | $- 1223.44 + 221.40*Temp + 3.89*DCO^2 - 513*pH - 97.90*Sali - 17.44*DO + 172.98*NO_3^- + 10.95*SS - 3.43*Cond + 2.51*Turb - 3.02*Col - 1045.91*NH_4^+ + 485.84*PO_4^{3-}$ | 1.06 | 0.43 |
| *V.alginolyticus* | 30 | $712.10 - 15.27*Temp + 2.10*DCO^2 - 116.98*pH - 15.30*Sali + 5.93*DO + 39.56*NO_3^- + 5.84*SS - 0.19*Cond - 0.61*Turb - 0.45*Col - 13.11*NH_4^+ + 65.51*PO_4^{3-}$ | 1.15 | 0.45 |
| *V. fluvialis* | 30 | $1305.76 - 36.95*Temp + 1.68*DCO^2 - 49.85*pH - 3.07*Sali - 1.29*DO + 41.01*NO_3^- + 2.72*SS + 0.02*Cond + 0.99*Turb - 0.58*Col - 185.26*NH_4^+ - 53.26*PO_4^{3-}$ | 0.49 | 0.26 |

50% of the total variation. The physicochemical parameters explain at 43% ($r^2 = 0.43$) the distribution of the abundances of *V. parahaemolyticus*, at 45% ($r^2 = 0.45$) the distribution of abundances of *V. alginolyticus* and at 26% ($r^2 = 0.26$) for *V. fluvialis*.

## DISCUSSION

The results of the physicochemical parameters show temporal variations. Overall, the water taken from the landing stages had temperatures that vary very little around an average of 29.94 ± 0.98°C. These temperatures are compatible with the activity of isolated microorganisms which are all mesophilic and promote the dissolution of gases and salts in water. Studies have shown that some pathogens grow extremely well at a mesophilic temperature range of 15 to 45°C for most strains (Vezzulli et al., 2016; Brenzinger et al., 2019). The surface water in the city of Douala is significantly warmer,

this could be explained by the fact that the city of Douala is closer to the sea and there is a high concentration of industrial activities and a strong urbanization of the watershed which exposes the water to solar rays. These observations are consistent with some studies in the same Wouri River (Tchakonté et al., 2014). Regarding water pH, it varies from one campaign to another, increases slightly between May and July. The slightly basic tendency of the pH was same in all the studical stations. The absence of significant differences in pH between the different studied stations would reflect the nature of the pedological substratum of the coastal region, which is the same everywhere. Indeed, the characteristic soils of this region are acidic and rich in iron hydroxide and alumina (Asaah et al., 2006). However, the monthly fluctuations in this parameter would probably be due to the influence of precipitation, industrial and urban discharges (Tamsa et al., 2021). Furthermore, the concentration of dissolved carbon dioxide in all the sites was relatively high. These high dissolved $CO_2$ contents should normally lead to acidic waters due to the formation of carbonic acid following the reaction of $CO_2$ with water.

The average salinity obtained for all the months and for all the stations was 5.69 ± 0.64 psu) this would therefore be due to a compensation of these contents by carbonic acid formed. In addition, regions subjected to relatively high temperatures experience water losses through evapotranspiration, which indirectly influence the salinity content of their waters (NOAA, 2014). The data obtained from oxygen contents showed relatively high oxygen saturation percentage values (65%) in June corresponding to the rainy month. During heavy rains, passive diffusion at the air-water interface by agitation of the water promotes its reoxygenation (Ginet and Decou, 1997). In addition, these variations would be directly linked to the seasonal variations in water temperature which condition the process of oxygen solubility. Suspended solids contents were relatively high in most stations throughout the sampling period. In fact, during the rainy season, pollutants and garbage removed by dredging on the watersheds are washed away by runoff (Koji et al., 2017). The parameter average for all stations is 28.97 ± 14.86 mg/L. Surface water with a Suspended Solids concentration between 14 and 24 mg/L is of questionable quality (Hébert and Légaré, 2000). This doubt of the quality of the water in these landing stages is reinforced by the results of the survey carried out among residents. Indeed, it results from this survey that an average quantity of 1 tonne 563 kg of waste is produced and dumped by the respondents per day in the waters of the said landing stages. Turbidity remained almost constant in all the stations during the first four months of the study and dropped sharply in June significantly ($p \leq 0.05$). This drop would be due to the dilution of the water in the landing stages by the heavy rains recorded between the end of May and the beginning of June.

Turbidity, Suspended Solids and color values were significantly correlated throughout the study period. Water is more turbid and colored when the density of the particles in suspension are higher (Rodier et al., 2009; Koji et al., 2017; Tamsa Arfao et al., 2021). Moreover, water color entirely results from the extraction of the organic matters in decomposition, as well as the dissolution of some ions as iron, the manganese and the copper (Olanezuk-Neyman and Bray, 2000 ; Signe et al., 2015).

The average phosphate obtained from the entire sample was 0.52 ± 0.43 mg/L. Organic pollution is perceptible when the orthophosphate content is greater than 0.5 mg/L, on the basis of this it can be affirmed that the waters of the landing stages are polluted (Tamsa et al., 2021). The ammonium ions contents were generally high. The average content of this parameter was 0.30 ± 0.15 mg/L for this study. Ammonium ion contents of the order of 0.5 to 1 mg/L of $NH_4^+$ in surface waters suggest sources of pollution located upstream and concentrations greater than 0.3 mg/L of $NH_4^+$ testify to significant organic pollution (Rodier et al., 2009). The nitrate values for the whole sampling period ranged from 2 to 13.22 mg/L with an average of 7.92 ± 3.28 mg/L. The high levels of mineral nitrogen ($NH_4^+$ and $NO_3^-$) and orthophosphates in the water at the various stations reflect the strong mineralization of the water and the anthropized nature of the Wouri watershed, which is distinguished by its significant input of allochthone organic matter, nitrogenous and / or phosphorus metabolic waste emanating from human activity. HAB provide an overall assessment of the microbial contamination of an aquatic environment by providing information on its autochthonous and allochthonous microflora (Larif et al., 2013; Abologo et al., 2016; Tamsa et al., 2021). Different species belonging to the *Vibrio* genus whose presence in water and food constitutes a danger to the health of the populations were isolated and identified from the water samples from all the wharves. These are *V. parahaemolyticus*, *V. cholerae*, *V. fluvialis* and *V. alginolyticus*. These species, known for their role in infectious diarrhea in humans, especially *V. cholerae* and *V. parahaemolyticus*, have been regularly isolated with spatial and temporal occurrence rates of 100% each.

The rates of occurrence of *V. fluvialis* and *V. alginolyticus*, not significantly different from the previous ones reached 100 and 80% spatially and temporally respectively. These high rates of isolation obtained both spatially and temporally justify the endogenous character of *Vibrio* for coastal and marine environments (Bonhomme, 2003) as well as the endemic character of vibrioses, with occurrence of cholera in Douala. According to some authors, isolation rates greater than 50% for an organism in a medium indicate that the latter is constant in this habitat (Dajoz, 2000). The analysis of the model of the dynamics of abundance of vibrioplankton made it possible to note a variation in the concentration of these bacterial cells under the effect of the water

temperature, organic matter, pH, dissolved oxygen and salinity. According to similar modeling work, the increase in the concentration of vibrioplankton is explained by temperature and salinity in surface water in Georgia (USA) (Turner et al., 2009). Many authors have reported on the temperature and salinity as determining factors in the regulation of growth and survival of *Vibrio*s in surface water (Wang and Gu, 2005; Johnson et al., 2012). Other authors have contributed to the elaboration of predictive model for *V. cholerae* on the basis of the variation of temperature and salinity (Louis et al., 2003; Huq et al., 2005). Positive correlation exhibited by temperature indicates its importance on distribution and abundance of *Vibrio* spp (Osunla et al., 2021). Indeed, bacteria of the *Vibrio* genus are able to adapt theirselves to low levels of salinity in water with high temperatures in the Wouri estuary. The regression models with HAB and *V. cholerae* explain more than 50% of the total variation and the physicochemical parameters explain at 43% the distribution of the abundances of *V. parahaemolyticus,* at 45% the distribution of abundances of *V. alginolyticus* and at 26% for *V. fluvialis*. In fact, several authors have widely published on the influence of temperature and salinity on the abundances of Vibrionaceae in coastal and estuarine waters (Johnson et al., 2012; Baker-Austin et al., 2013). Bacterial species of the genus *Vibrio* are often associated with aquatic environments with particular physical and chemical properties. Thus, those incriminated in epidemics have always been associated with sea water (saline). Somes authors shown that the distribution of *Vibrio* species positively and significantly correlated with turbidity, temperature, dissolved oxygen, pH, total dissolved solid, total suspended solid, electrical conductivity and salinity (Osunla et al., 2021). The strong positive association of water temperature on the occurrence of *V. cholerae*, for example in the Akwa landing stage, confirms this relationship. In addition, the highest concentration of vibrioplankton in urban waters can be explained by the ability of these bacteria to adapt to organic pollution. According to some authors, vibrios are able to reduce forms of nitrogen in water to take advantage of urban pollution (Grimes et al., 2009).

## Conclusion

At the end of this study, it emerges that the waters of the wharves are home to a large community of heterotrophic aerobic mesophilic bacteria including 4 species of the *Vibrio* genera, namely *V. cholerae*, *V. parahaemolyticus*, *V. alginolyticus*, and *V. fluvialis*. *V. cholerae* was the most frequently isolated and also the most abundant. The isolation of these bacteria in the water points studied can be source of real public health problems for the using populations, and are incriminated in many diarrheal diseases, food poisoning and gastroenteritis would justify the outbreaks of vibriosis recorded in Douala for several years. The Multiple linear regression models and the visualization of corrgram revealed that the diversity and abundance dynamics of these germs is strongly influenced by nitrates, salinity, dissolved carborn dioxyde and ammonium ions.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

### REFERENCES


Abba SI, Hadi SJ, Abdullahi J (2017). River water modelling prediction using multi linear regression, artificial neural network, and adaptive neuro-fuzzy inference system. Procedia Computer Science 120:75-82.

Abologo AL, Essomba NC, Banock L, Bilong BC (2016). Qualité microbiologique des eaux des fleuves Nyong et Wouri (Cameroun) et impact potentiel sur la santé des populations riveraines. International Journal of Biological Chemical Sciences 10(3):1235-1249.

Alizadeh MJ, Kavianpour MR, Danesh M, Adolf J, Shamshirband S, Chau KW (2018). Effect of river flow on the quality of estuarine and coastal waters using machine learning models. Engineering Applications of Computational Fluid Mechanics 12(1):810-823.

American Public Health Association (APHA) (2009). Standard Methods for the Examination of Water and Wastewater. America Public Health Association, APHA-AWWAWPCF (Eds.), Pennsylvania, Washington.

Asaah VA, Abimbola AF, Suh CE (2006). Heavy Metal Concentration and Distribution in Surface Soils of the Bassa Industrial Zone 1 Douala Cameroon. Arabian Journal for Science and Engineering 31(2):147-158.

Baker-Austin C, Trinanes JA, Taylor NGH, Hartnell R, Siitonen A, Martinez UJ (2013). Emerging Vibrio risk at high latitudes in response to ocean warming. Nature Climate Change 3(1):73-77.

Beatley T, Browner DJ, Schwab AK (1994). An Introduction to Coastal Zone Management Island Press, Washington D.C. P 98.

Ben MA, Chahlaoui A, Rour E, Chahboune M (2014). Diversité taxonomique et structure de la macrofaune benthique des eaux superficielles de l'oued khoumane. Journal of Materials and Environmental Science 5(1):183-198.

Bonhomme AJ (2003). Les bactéries du genre *Vibrio* et la santé publique vétérinaire. Doctorat vétérinaire. Ecole Nationale Vétérinaire d'Alfort. P 109.

Brenzinger S, Van der Aart LT, Van Wezel GP, Lacroix JM, Glatter T, Briegel A (2019). Structural and proteomic changes in viable but non-culturable *Vibrio cholerae*. Frontier in Microbiology 10:793.

Castaneda CM, Sedas VP, Borunda EO, Reynoso FL (2005). Influence of water temperature and salinity on seasonal occurrences of *Vibrio cholerae* and enteric bacteria in oyster-producing areas of Veracruz. Marine Pollution Bulletin 50(12):1641-1648.

Chen W, Liu W (2015). Water Quality Modeling in Reservoirs Using Multivariate Linear Regression and Two Neural Network Models. Advances in Artificial Neural Systems ID 521721. P 12.

Conley DJ (2000). Biogeochemical nutrient cycles and nutriment strategies. Hydrobiologia 410:87-96.

Dajoz R (2000). Précis d'Ecologie. 7ème édition, Dunod, Paris, France P 615.

Ginet R, Decou V (1997). Initiation à la biologie et à l'écologie souterraines. Ed. Delarge Paris P 345.

Giresse P, Megope-foonde J, Ngueutchoua G, Aloisi J, Kuete M (1996). Carte sédimentologique du plateau continental du Cameroun. Rapport de l'Institut Français de recherche scientifique pour le développement en coopération. ORSTOM (ed).

Gonzáles HE, Giesecke R, Vargas CA, Pavez M, Iriarte JL, Santibáñez P, Castro L, Escribano R, Pagès F (2004). Carbon cycling through the pelagic foodweb in the northern Humboldt Current off Chile (23°S). ICES Journal of Marine Science 61(4):572-584.

Grimes DJ, Johnson CN, Dillon KS, Flowers AR, Noriea NF, Berutti T (2009). What Genomic Sequence Information Has Revealed About

*Vibrio* Ecology in the Ocean-A Review. Microbial Ecologie 58(3):447-460.

Hamuna B, Tanjung RHP, Suwito, Maury HK (2018). Study of sea water quality and pollution in¬dex based on physical-chemical parameters in De¬papre District waters, Jayapura. Jurnal Ilmu Ling¬kungan 16(1):35-43. (in Indonesian).

Hébert S, Légaré S (2000). Suivi de la qualité des rivières et petits cours d'eau. Direction du suivi de l'état de l'environnement, ministère de l'Environnement, Québec, envirodoq No ENV-2001-0141, rapport N° QE-123, P 24.

Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST (2000). Bergey's manual of determinative bacteriology. Williams and Wilkins, ed Philadelphia.

Huq A, Sack RB, Nizam A, Longini IM, Nair GB, Ali A, Morris Jr JG, Khan MNH, Siddique AK, Yunus M, Albert MJ, Sack DA, Colwell RR (2005). Critical factors influencing the occurrence of Vibrio cholerae in the environment of Bangladesh. Applied and Environmental Microbiology 71(8):4645-4654.

Johnson CN, Bowers JC, Griffitt KJ, Molina V, Clostio RW, Shaofeng P, Laws E, Paranjpye RN, Strom MS, Chen A, Hasan NA, Huq A, Noriea III NF, Grimes DJ, Colwell RR (2012). Ecology of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington (United States). Applied Environmental Microbiology 78(20):7249-7257.

Koji E, Noah EO, Onana FM, Tchakonté S, Lontsi DC, Tamsa AA, Bricheux G, Sime-Ngando T, Nola M (2017). Influence of Anthropogenic Pollution on the Abundance Dynamics of Some Freshwater Invertebrates in the Coastal Area of Cameroon. Journal of Environmental Protection 8(7):810-829.

Larif M, Soulaymani A, Hnach M, El Midaoui A (2013). Contamination spatio-temporelle d'origine hydrique de l'oued Boufekrane dans la région de Meknès-Tafilalt (Maroc). International Journal of Biological and Chemical Sciences 7(1):172-184.

Louis VR, Russek-Cohen E, Choopun N, Rivera ING, Gangle B, Jiang SC, Rubin A, Patz JA, Huq A, Colwell RR (2003). Predictability of *Vibrio cholerae* in Chesapeake Bay. Environmental Microbiology 69(5):2773-2785.

Niang I, Naï G, Folorunsho R, Diop M, Sow M, Trawally D, Faye S, Bihibindi A, Diop N, Karibuhoye C (2012). Guide sur les options d'adaptation en zones côtières à l'intention des décideurs. UNESCO/COI. P 54.

NOAA (2014). Oil Spills in Mangroves: Planning and Response Considerations (Maryland: Office of Response and Restoration) P 96.

Nola M, Njiné T, Djuikom E, Sikati FV (2002). Faecal coliforms and faecal streptococci community in the underground water in an equatorial area in Cameroon (Central Africa): the importance of some environmental chemical factors. Water Research 36(13):3289-3297.

Olanezuk-Neyman K, Bray R (2000). The Role of Physico-Chemical and Biological Processes in Manganese and Ammonia Nitrogen Removal from Groundwater. Polish Journal of Environmental Studies 9(2):91-96.

Osunla CA, Okoh AI (2017). Vibrio pathogens: A public health concern in rural water resources in Sub-Saharan Africa. International Journal of Environmental Research and Public Health 14(10):1188.

Osunla AC, Abioye OE, Okoh AI (2021). Distribution and Public Health Significance of Vibrio Pathogens Recovered from Selected Treated Effluents in the Eastern Cape Province, South Africa. Water 13:932.

Parmar KS, Bhardwaj R (2015). River Water Prediction Modeling Using Neural Networks, Fuzzy and Wavelet Coupled Model. Water Resources Management 29(1):17-33.

Pravakar M, Panda US, Pradhan U, Kumar CS, Naik S, Begum M, Ishwarya J (2015). Coastal water quality monitoring and modelling off Chennai city. Procedia Engineering 116:955-962.

Rodier J, Legube B, Marlet N, Brunet R (2009). L'analyse de l'eau. 9e édition, DUNOD, Paris. P 1579.

Signe JM, Lontsi CD, Njoya AM, Eheth JS, Tchakonté S, Tamsa AA, Ateba HB, Bricheux G, Nola M, Sime-Ngando T (2015). Assessment of the potential effect of somme streams properties on the isolated *Aeromonas hydrophila* strains susceptibility against some β-Lactams and Sulfamids. Research in Biotechnology 6(5):33-44.

Suchel JB (1972). Le climat du Cameroun. Thèse de Doctorat 3eme cycle, Université de Bordeaux III. P 186.

Tamsa AA, Onana FM, Koji E, Moungang LM, Noah EOV, Tchuimaleu EJB, Tchakonté S, Njoya AM, Sime-Ngando T, Nola M (2021). Using Principal Component Analysis to Assess Water Quality from the Landing Stages in Coastal Region. American Journal of Water Resources 9(1):23-31. doi: 10.12691/ajwr-9-1-4.

Tanjung RHP, Hamuna B, Alianto (2019). Assessment of Water Quality and Pollution Index in Coastal Waters of Mimika, Indonesia. Journal of Ecological Engineering 20(2):87-94.

Tchakonté S, Ajeagah GA, Diomandé D, Camara AI, Konan KM, Ngassam P (2014). Impact of anthropogenic activities on water quality and Freshwater Shrimps diversity and distribution in five rivers in Douala, Cameroon. Journal of Biodiversity and Environmental Sciences 4(2):183-194.

Tchakonté S (2016). Diversite et structure des peuplements de macroinvertebres benthiques des cours d'eau urbains et periurbains de Douala (Cameroun). These de Doctorat/PhD, Universite de Yaounde P 306.

Turner JW, Brooks G, Dana C, Erin KL (2009). Plankton composition and environmental factors contribute to *Vibrio* seasonality. Multidisciplinary Journal of Microbial Ecology 3(9):1082-1092.

Vezzulli L, Grande C, Reid PC, Hélaouët P, Edwards M, Höfle MG, Brettar I, Colwell RR, Pruzzo C (2016). Climate influence on Vibrio and associated human diseases during the past half-century in the coastal North Atlantic. Proceeding of the National Academic of Sciences 113(34):5062-5071.

Wang YL, Gu J-D (2005). Influence of temperature, salinity and pH on the growth of environmental *Aeromonas* and *Vibrio* species isolated from Mai Po and the Inner Deep Bay Nature Reserve Ramsar Site of Hong Kong. Journal of Basic Microbiology 45(1):83-93.

Zhong L, Chen J, Zhang XH, Jiang YA (2009). Entry of *Vibrio cincinnatiensis* into viable but non culturable state and its resuscitation. Letters in Applied Microbiology 48(2):247-252.

Vol. 15(6), pp. 318-324, June, 2021
DOI: 10.5897/AJMR2021.9528
Article Number: 5899D3867055
ISSN: 1996-0808
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# Probiotic properties of Lactobacilli isolated from human milk and their inhibitory effect on gastrointestinal pathogenic bacteria

**Mohammad Mehdi Soltan Dallal[1,2], Niloofar Moghadam Maragheh[1], Zahra Rajabi[2], Mohammad Bagher Hosseini[3], Moslem Papizadeh[4]*, Gholamreza Hassanpour[5], Sara Sharifi Yazdi[6], Mojtaba Memariani[7], Shabnam Haghighat Khajavi[8], Seyedeh Masoomeh Abrichamchian langaroodi[9], Hedroosha Molla Agha Mirzaei[2] and Mehrnaz Mirzababaei[1]**

[1]Division of Food Microbiology, Department of Pathobiology, School of Public health, Tehran University of Medical Sciences, Tehran, Iran.
[2]Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[3]Pediatric Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[4]Research Assistant Microbiology Research Center, Pasteur Institute of Iran, Tehran, Iran.
[5]Parasitology, Center for Research of Endemic Parasites of Iran, Tehran University of Medical Sciences Tehran, Iran.
[6]School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[7]Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[8]Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[9]Pathobiology Laboratory Center, No17, Gharani Alley, Valiasr Ave, Tehran, Iran.

Received 12 March, 2021; Accepted 1 June, 2021

**Breast feeding has critical effects on the newborns and either mother's health. Some of such health-improving effects of the mother's milk is associated with the beneficial microbes, lactic acid bacteria (LAB), which are normally present in the mother's milk. Thus, human milk-associated lactobacilli were isolated in this study and some of their probiotic properties was investigated. Afterwards, *Lactobacillus* strains were screened for low pH and bile acids tolerance. Molecular identification was carried out using 16SrDNA and polymerase chain reaction (PCR). Antibiotic resistance was evaluated with disk diffusion assay and the inhibitory effect of isolates on pathogenic bacteria was examined with well assay and zone inhibition. Isolation experiments resulted in 122 human milk- associated lactobacilli belonging to 12 species. The most dominant species was *Lactobacillus casei* followed by *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus gasser*, respectively. Screening for probiotic properties showed that 19 isolates, belonging to, *Lactobacillus*, have interesting probiotic characteristics. The most prevalent antibiotic resistance was observed in case of vancomycin (63.15%) and no drug resistance was detected for chloramphenicol, penicillin, rifampin (0%). Three *Lactobacillus* strains, designated as L4, L14 and L16, were found as potential probiotic strains since they have indicated promising inhibitory effects against the studied pathogenic bacterial strains. Our results shed light on the considerable diversity of lactobacilli in human breast milk. Furthermore, the candidate probiotic strains detected in this research might be used as potential probiotic strains.**

**Key words:** Breast milk, *Lactobacillus*, probiotics, inhibitory effect, polymerase chain reaction.

## INTRODUCTION

The name probiotic stemmed from the Greek “pro bios” meaning literally “for life” (Soccol et al., 2010). Breastfeeding, as the main source of infants’ nutrition, affects the development of the microbiota in the gastrointestinal tract (Martín et al., 2005). It is estimated that newborns consume around 0.8 L/day breast milk which contains $10^5$-$10^7$ colony forming units (CFUs) of milk microorganisms (Olivares et al., 2006). Breast milk microbiota comprises an array of microbial groups including the genera of lactic acid bacteria (LAB) (Damaceno et al., 2017).

The most pervasive probiotic species are represented by the following genera: *Lactobacillus*, *Streptococcus*, and *Bifidobacterium*, but other microorganisms including enterococci and yeasts (e.g. *Saccharomyces boulardi*) have also been exploited as probiotics (Soccol et al., 2010). Among the organisms occurring in milk, some species including *Lactobacillus salivarius*, *Lactobacillus fermentum*, *Lactobacillus gasseri*, *Bifidobacterium breve*, *Bifidobacterium adolescentis*, and *Bifidobacterium longum* have shown potential to enhance mother and infant health (Ruiz et al., 2019). Lactobacilli are aerotolerant, Gram-positive, non-motile, non-spore-forming, catalase and oxidase negative bacilli that are mainly fermentative bacteria. They consume simple sugars and produce lactate as their main metabolic product. Many species of *Lactobacillus* have long been known as antagonists of pathogenic bacteria (Wadher et al., 2010). Lactobacillus species produce significant amounts of lactic acid which causes a detectable pH drop. The acidification influences the growth of various microorganisms in human gut. Furthermore, lactic acid accumulation affects the fungal growth. The other antimicrobial property of lactobacilli originates from the nisin, the small peptides produced by these small-genome bacteria (Lindgren and Dobrogosz, 1990). The protective effects of lactobacilli and other probiotics against various intestinal pathogens have been extensively studied over the past decades, leading to commercial usage of lactobacilli in probiotic market as an effective therapy for many gastrointestinal diseases for not only human, but also farm animals (Gronlund et al., 2000).

Gram-negative intestinal bacteria, especially genera *Salmonella* and *Shigella* as well as *Escherichia coli*, are known as the major food-borne infection and diarrhea in many developing countries including Iran (Fardsanei et al., 2018; Talebreza et al., 2016; Memariani et al., 2014). To make matters worse, the rise of antibiotic resistance is a growing concern worldwide. Probiotic may reduce the rate of development of drug resistant pathogenic strains secondary to widespread and injudicious antibiotic use (Soltan Dallal et al., 2016a).

Breast milk can be regarded as one of the main resources for potential probiotic candidates. Many studies showed the potential diversity of bacterial species of breast milk (Collado et al., 2009; Jost et al., 2013; Zimmermann and Curtis, 2020). LAB and *Bifidobacterium* make up to 85% of the entire bacterial population of the intestinal microflora (Soccol et al., 2010). Considering the positive effects of breast milk on the microflora of the gastrointestinal tract of newborns, lactobacilli were isolated from breast milk samples in this study. Furthermore, the capability of the *Lactobacillus* isolates to inhibit the gastrointestinal pathogens was investigated.

## MATERIALS AND METHODS

### Sampling

One hundred breast milk samples, around 3 ml, were collected from volunteer breast feeder mothers who were between 22-36 years old. Volunteers had no antibiotic usage 2 weeks prior to sampling. Milk samples were collected in sterile conditions and promptly transported to the laboratory under refrigerated conditions. Briefly, before sample collection, wearing sterile gloves, the women cleaned the nipple and surrounding area with an alcohol swab to minimize the presence of skin bacteria. The first few drops of manually expressed milk were also discarded (Li et al., 2017).

### Enrichment and isolation

Enrichments were performed in MRS medium with 10 times dilution. After 48 h of incubation at 37°C, aliquots were streaked on MRS agar plates which were also incubated at the above-mentioned conditions. The emerged colonies were further purified and catalase negative Gram-positive colonies were subjected to biochemical and morphological characterizations (Soltan Dallal et al., 2016b). *L. plantarum* strain PTCC 1058 was used as the positive control.

### Carbohydrate fermentation

The MRS broth medium (pH = 8) with no glucose and neither meat extract, containing 0.004% chloramphenicol red was used for carbohydrate fermentation assays. Such experiments were performed using 13 sugars with 0.5% (*w*/*v*) concentration (Marroki et al., 2011). Microplates were inoculated with a dense suspension of the given isolates and coverage was performed by autoclave-sterilized paraffin to establish the anaerobic conditions. Incubation at 35°C was performed for 5 days and a color change from violet to red was assumed as carbohydrate fermentation positive (Davoodabadi et al., 2015).

*Corresponding author. E-mail: msoltandallal@gmail.com. Tel: +98-21-: 88992971.

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#### Tolerance to acid and bile salts

To assess the tolerance of the isolates to acidic conditions, 1 ml of a 24 h culture of the isolate was inoculated into 9 ml of PBS buffer (pH = 2.5) and after 3 h of incubation at 37°C, the viability of the isolate was observed (Soltan Dallal et al., 2016c). Acid-resistant isolates were further screened for resistance against bile salts. Two test tubes, one containing 9 ml MRS broth with 0.3% (*w*/*v*) bile salt Oxgall and the other one containing 9 ml MRS broth without Oxgall (negative control) were defined for each acid-resistant isolate. Test tubes were inoculated with 90 µl of cultures of given isolates and incubated at 37°C. The optical density ($OD_{600}$) of the test tubes was assessed at inoculation time and either after 8 h of incubation (Sharifi et al., 2017).

#### Antimicrobial properties

The antimicrobial properties of the acid-bile resistant lactobacilli against *E. coli* O157: H7 (ATCC 43894), *Salmonella enteritidis* (ATCC 14028), *Shigella sonnei* (ATCC 25931), *Yersinia enterocolitica* (ATCC 23715), methicillin-resistant *Staphylococcus aureus* (ATCC 43300) were determined using well diffusion method as described previously (Rammelsberg and Radler, 1990). The pathogenic bacteria were cultured first in Luria-Bertani (LB) broth (Merck Co. Darmstadt, Germany) for 24 h at 37°C and their concentration was adjusted to $10^7$ CFUs/ml, then were cultured on the surface of nutrient agar medium (Merck Co. Darmstadt, Germany) employing well diffusion agar method. After growing on MRS broth liquid culture mediums in candle jar at 37°C for 20 h, centrifugation was performed at 13,000 rpm for 10 min. The culture supernatant was then collected. One hundred microliters of liquid on culture was added to each well of the nutrient agar plate using well diffusion agar method, and the plates were then incubated for 15 h at 37°C. After the incubation time, the diameter of the growth inhibition zone around the wells was measured. Isolates with no growth halo diameter or < 11 mm were defined as negative, 11-16 mm as a moderate inhibitor (+), 17-12 mm as strong inhibitor (++), and ≥ 23 mm as a very strong inhibitor (+++) (Jomehzadeh et al., 2020). *L. rhamnosus* GG was used as a positive control and MRS sterile broth was used as a negative control (Lashani et al., 2018).

#### DNA extraction and molecular methods

DNA extraction of *Lactobacillus* isolates for the molecular analysis was performed according to the method of Chandok et al. (2015) with some modifications. First, pure culture of *Lactobacillus* isolates was prepared on MRS agar medium and 2 to 3 colonies were dissolved in 50 µl of STE (Sodium Chloride-Tris-EDTA) solution. The suspension was placed in a water bath at 96°C for 10 min (Boiling method), then the suspension was centrifuged at 13,000 rpm for 3 min and the supernatant was used for PCR (Soltan Dallal et al., 2017a).

PCR for 16S rDNA gene was performed to confirm the identification of *Lactobacillus* isolates with probiotic potency. This reaction was performed with 12.5 µl of Master Mix, Forward and Reverse primers of 0.3 µl each, 2 µl of DNA, and final volume up to 25 µl by distilled water. For PCR reaction, primers 27F (5′-CTCGTTGCGGGACTTAA-3′) and 1522R (5′-GCAGCAGTAGGGAATCTTC-3′) were used (Soltan Dallal et al., 2017b). PCR was performed with initial denaturation at 94°C for 2 min, denaturation at 94°C for 30 s, binding at 55°C for 1 min, elongation at 72°C for 1 min, and final elongation at 72°C. It was performed for 10 min during 35 cycles. Amplified DNA fragments were observed as single bands with a size of 757 bp by agarose gel electrophoresis (1.5% *w*/*v*) stained with ethidium bromide (Figure 1).

## RESULTS

### Isolated bacterial species

Out of 100 milk samples, 97 samples were positive for colony growth and the presence of *bacillus* and cocci lactic acid bacteria. A total of 122 *Lactobacillus* strains were isolated from breast milk samples. To identify *Lactobacillus* species, carbohydrate fermentation tests, growth test at 15 and 45°, arginine hydrolysis and glucose gas production were used. Among 122 isolates, 111 *Lactobacillus* species (90.99%) were identified by biochemical tests and 11 isolates (9.01%) were not identified during biochemical phenotypic tests. A total of 12 different species of *Lactobacillus* were identified in breast milk samples (Table 1). These species included: *L. casei*, *L. fermentum*, *L. plantarum*, *L. gasseri*, *L. curvatus*, *L. paracasei*, *L. acidophilus*, *L. reuteri*, *L. brevis*, *L. pentosus*, and *L. rhamnosus*. Among these species, the most dominant species was *L. casei* (*n* = 43; 35.25%).

### Investigation of probiotic potency of isolates

Out of 122 *Lactobacillus* isolates studied, 22 isolates withstood acidic conditions with pH 2. In the next step, these 22 acid-resistant strains were examined for resistance to 0.3% oxgall bile salt. Of these isolates, 19 strains were finally resistant to both acidic and bile salt conditions and these strains were confirmed as strains with probiotic potential. A total of 19 *Lactobacillus* strains that showed resistance to acid and bile were *L. casei* (*n* = 5), *L. brevis* (*n* = 5), *L. actobacillus* (*n* = 2), *L. fermentum* (*n* = 2), *L. paracasei* (*n* = 2), *L. rhamnosus* (*n* = 2), and *L. reuteri* (*n* = 1).

### Evaluation of antimicrobial effects of probiotic lactobacilli against gastrointestinal pathogens

Antimicrobial activity of 19 acid-resistant lactobacilli isolates against a number of gastrointestinal pathogens was examined (Figure 2). Antimicrobial activity of all 19 lactobacilli isolates was performed without temperature and enzymatic treatments. Out of 19 acid- and bile-resistant lactobacilli isolates, 10 strains (52.63%) were able to inhibit the growth of *E. coli* O157 H7, of which 6 strains had a strong inhibitory effect and 4 strains had a moderate inhibitory effect and 9 isolates did not have any inhibitory effect. The results showed that 12 isolates of probiotic lactobacilli (63.16%) were able to inhibit the growth of *Salmonella*, of which 9 isolates strongly or very strongly inhibited *Salmonella*. 3 isolates had moderate inhibitory effect and 7 isolates had no inhibitory effect. All 19 probiotic isolates (100%) were able to inhibit *Shigella* growth, of which 9 strains had strong effect, 6 strains had very strong effect and 4 strains had moderate inhibitory effect. 7 probiotic strains (36.84%) had inhibitory effect of

**Table 1.** The list of *Lactobacillus* species identified in this study. The number (*n*) and percentages (%) of each species are represented.

| S/N | Strains | *n* | % |
|---|---|---|---|
| 1 | *L. casei* | 43 | 35.25 |
| 2 | *L. fermentum* | 18 | 14.75 |
| 3 | *L. plantarum* | 12 | 9.84 |
| 4 | *L. gasseri* | 10 | 8.20 |
| 5 | *L. paracasei* | 6 | 4.92 |
| 6 | *L. acidophilus* | 5 | 4.09 |
| 7 | *L. brevis* | 5 | 4.09 |
| 8 | *L. reuteri* | 4 | 3.28 |
| 9 | *L. curvatus* | 4 | 3.28 |
| 10 | *L. rhamnosus* | 2 | 1.64 |
| 11 | *L. pentosus* | 1 | 0.82 |
| 12 | *L. jenseni* | 1 | 0.82 |
| 13 | *Non identified* | 11 | 9.02 |
| | Total | 122 | 100 |

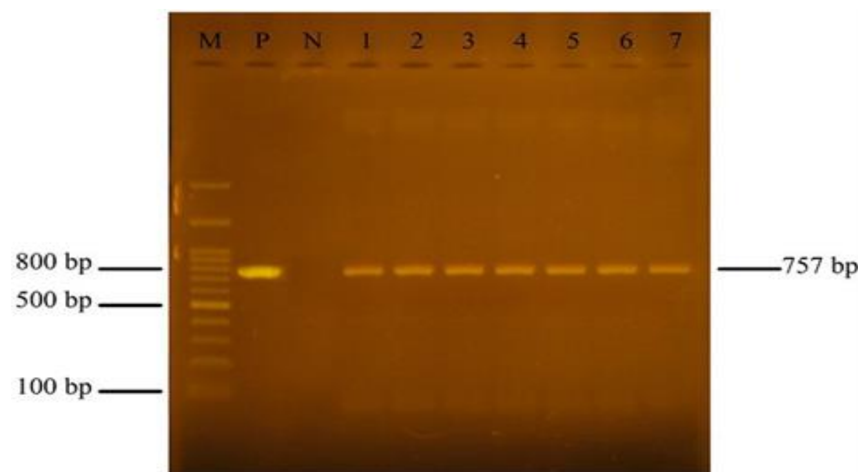


**Figure 1.** 16S RNA Gene Polymerase Chain Reaction Products (757 bp). Lane M, 100 bp DNA marker (Sinaclon, Iran); Lane N, Negative control; Lane P, Positive control (*L. acidophilus* ATCC 4356); Lanes 1-7, *Lactobacillus* isolates. For the negative control PCR was conducted without adding DNA.

*S. aureus,* of which 5 strains had a strong inhibitory effect and 2 strains had a moderate inhibitory effect. The other 12 strains of *Lactobacillus* had no inhibitory effect on the growth of *S. aureus*. 11 strains of probiotic Lactobacillus isolates (57.89%) had a strong inhibitory effect on the growth of *Yersinia* and 8 strains had no inhibitory effect on growth. Among 19 probiotic lactobacilli strains, 3 strains L4, L14 and L16 had strong inhibitory effect on all gastrointestinal pathogens tested.

## DISCUSSION

The microflora of infants, especially lactic bacteria such as *Lactobacillus* and *Bifidobacterium*, plays a pivotal role in gastrointestinal tract in creating a proper balance of intestinal microorganisms, as well as beneficial properties on infant health and immune system promotion, in particular cellular immunity (Galdeano et al., 2019). The baby's gastrointestinal tract is sterile until birth, and microbial colonization begins gradually after this event. Microflora during infancy is under the influence of various factors including type of delivery (cesarean section or normal), environmental health, use of antibiotics by mother or baby, climatic and geographical conditions, type of feeding (formula or breast milk), and intestinal maturity (Navarro-Tapia et al., 2020). Lactobacilli are a heterogeneous group of lactic acid bacteria present in the

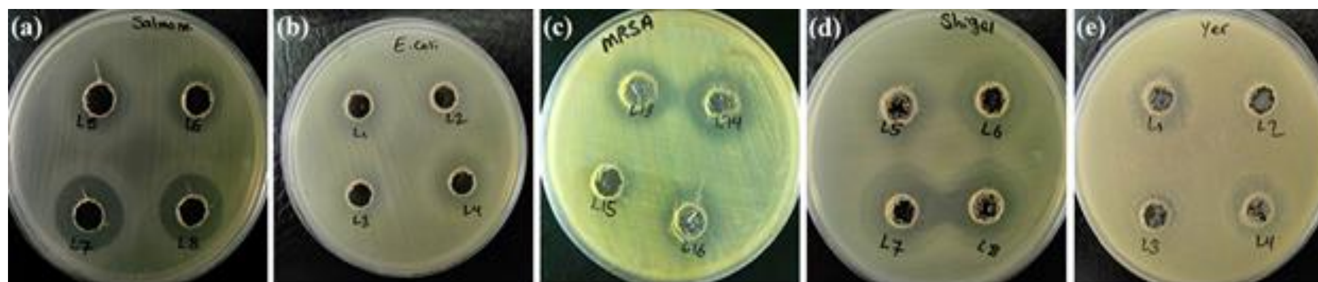


**Figure 2.** Antimicrobial activity of probiotic lactobacilli against different bacterial pathogens. Panels a, b, c, d, and e represent *Salmonella enteritidis* ATCC 14028, *Escherichia coli* O157: H7(ATCC 43894), *Staphylococcus aureus* ATCC 43300, *Shigella sonnei* ATCC 25931, and *Yersinia enterocolitica* ATCC 23715, respectively.

intestinal flora of healthy people (Davoodabadi et al., 2015). These symbionts are part of the natural flora of the reproductive system of humans and animals. Breast milk is a continuous and useful food source for the development and creation of microorganisms in the baby's intestine, thereby identifying strains with probiotic properties from this source is of importance (Martín et al., 2005).

In this study, lactobacilli isolated from 100 samples of breast milk in which *L. casei* (35.2%) was the most abundant species. Bacteria growth was better during the isolation process under microaerophilic conditions. Therefore, it is recommended to use microaerophilic conditions for optimal isolation of these bacteria. Similar results have been reported by Brigidi et al. (2001).

In a study of conducted by Xanthopoulos et al. (1999), MRS was used to isolate lactobacilli at 37°C, which is in accordance with the method used in the present study. Yuki et al. (1999) also used lactitol-LBS-vancomycin agar (LLV) agar to isolate lactobacilli. The results of these studies show that in order to isolate lactobacilli, diverse but rich and slightly acidic culture media can be used (Xanthopoulos et al., 1999; Yuki et al., 1999).

In the present study, out of 122 strains, 11 strains (9%) could not be identified by phenotypic methods of carbohydrate fermentation. The difference between these bacteria in sugar fermentation and non-compliance with the standard tables could be due to mutations in one or more genes related to intermediate enzymes in fermentation of sugars that have mutated or deleted during evolution, change of environmental conditions and lack of need for the relevant gene (Hedberg et al., 2008).

Given that the identification of bacteria at the species level based on phenotypic characteristics is not accurate due to large changes in fermentation pattern, the study of ribosomal DNA gene sequences is a superior approach for phylogenetic comparison (Urbaniak et al., 2016). Soto et al. (2014) examined 160 samples of breast milk for the presence of *Lactobacillus* and *Bifidobacterium* and from 40.91% of the samples succeeded in isolating lactobacilli, the predominant species of which included *L. salivarius*, *L. fermentum*, and *L. gasseri*, respectively. Additionally, the isolation rate of lactobacilli and bifidobacteria was significantly lower in mothers treated with antibiotics than in healthy mothers. The above study clearly states the importance of the use of antibiotics and the effectiveness of breast milk flora from the digestive flora during lactation. In the present study, the isolation of lactobacilli from breast milk samples was 97%. In our study, in order to isolate maximum lactobacilli in breast milk flora, mothers who were treated with antibiotics for two weeks before sampling were excluded (Soto et al., 2014).

In a study which conducted on 7 mothers, Jost et al. (2013) isolated staphylococci, streptococci, bifidobacteria, and lactobacilli over three periods of 3-6, 14-9, and 25-30 days postpartum. Of the lactobacilli, only *L. brevis* and *L. gasseri* was isolated from the studied individuals, while in the present study, *L. casei* and *L. fermentum* were the most dominant species. These differences can be attributed to the differences in the flora of breast milk in different lactation periods and various geographical areas (Jost et al., 2013).

In a study by Gomez-Gallego , *L. plantarum* species was solely isolated from milk samples lactobacilli, while in the current study, 12 different species of *Lactobacillus* were isolated. This difference in isolation can be justified by regional differences because the breast milk microbiome is affected by specific factors including genetics, health status, maternal nutrition and geographical location (Taghizadeh et al., 2017; Jiang et al., 2016). Urbaniak et al. (2016) isolated *Staphylococcus*, *Streptococcus*, and *Lactobacillus* strain from 39 Canadian breast milk samples using 16SrDNA, of which Staphylococci formed the dominant species with 31% prevalence rate (Urbaniak et al., 2016).

In another survey, Kumar et al. (2016) evaluated 80 breast milk samples from 4 different geographic regions in Europe (Spain and Finland), Africa (South Africa) and Asia (China). The results of this study showed that breast milk in different regions shows a completely different pattern in terms of the presence of microbial flora (Kumar et al., 2016). Moreover, the results of a study on 133 breast milk samples from various regions of Taiwan and China demonstrated that different stages of lactation are

effective in microbiome diversity and the composition of the microbial flora of breast milk can be different based on geographical regions (Li et al., 2017).

This study has some limitations, which should be addressed in future. For instance, in order to consider probiotics for human consumption, they should undergo *in vitro* and *in vivo* biosafety assessment. Biosafety assessment should take into account the nature of the organism being used, method of administration, level of exposure, health status of consumers and physiological functions they are called on to carried out (Sanders et al., 2010). The antibiotic resistance is also an important criterion for biosafety. The probiotic should not contain any transferable antibiotic resistance gene. Further investigation is also warranted for probiotic use in high risk human populations including severely immune-compromised individuals, neonates or hospitalized patients. Probiotics should not produce high amount of biogenic amines because these low molecular weight compounds can lead to various human ailments such as vomiting, hypertension, palpitations, and headache. In addition, the mucin degradation is an important criterion for biosafety assessment of probiotics. The probiotic should not degrade mucin (Kurkutia et al., 2019).

## Conclusion

The present study on the study of breast milk microflora showed that there are various species of Lactobacillus in the milk of healthy mothers. The reason for this diversity of *Lactobacillus* species in breast milk is probably due to the type of nutrition and diet, lifestyle, age, climatic and geographical conditions, immune system status and many other factors.

Phenotypic identification methods based on biochemical properties alone are not sufficient to properly identify and differentiate all of the bacterial species; thereby more accurate techniques, in particular molecular methods, such as 16SrDNA gene sequencing are required for identification of various specific probiotic strains.

In our study, Lactobacillus species with good probiotic potential such as *L. casei* and *L. fermentum* were isolated, similar to other studies. Although various species of *Lactobacillus* were isolated from breast milk and even some species were dominant in terms of frequency, only few species showed probiotic properties. This study confirms that probiotic property is strain-dependent. Future studies should focus on the factors that may modulate the quantitative and qualitative composition of the breast milk microbiota.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors appreciate Microbiology Lab staff. This study was a part of a research project approved by the Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran (Contract No. 29352).

## REFERENCES

Brigidi P, Vitali B, Swennen E, Bazzocchi G, Matteuzzi D (2001). Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. Research in Microbiology 152(8):735-741.

Chandok H, Shah P, Akare UR, Hindala M, Bhadoriya SS, Ravi GV, Sharma V, Bandaru S, Rathore P, Nayarisseri A (2015). Screening, isolation and identification of probiotic producing *Lactobacillus acidophilus* strains EMBS081 & EMBS082 by 16S rRNA gene sequencing. Interdisciplinary Sciences: Computational Life Sciences 7(3):242-248.

Collado MC, Delgado S, Maldonado A, Rodríguez JM (2009). Assessment of the bacterial diversity of breast milk of healthy women by quantitative real-time PCR. Letters in applied microbiology 48(5):523-528.

Damaceno QS, Souza JP, Nicoli JR, Paula RL, Assis GB, Figueiredo HC, Azevedo V, Martins FS (2017). Evaluation of potential probiotics isolated from human milk and colostrum. Probiotics and antimicrobial proteins 9(4):371-379.

Davoodabadi A, Soltan Dallal MM, Lashani E, Tajabadi Ebrahimi M (2015). Antimicrobial activity of *Lactobacillus* spp. isolated from fecal flora of healthy breast-fed infants against diarrheagenic Escherichia coli. Jundishapur journal of microbiology 8(12):e27852.

Fardsanei F, Soltan Dallal MM, Douraghi M, Memariani H, Bakhshi B, Zahraei Salehi T, Nikkhahi F (2018). Antimicrobial resistance, virulence genes and genetic relatedness of Salmonella enterica serotype Enteritidis isolates recovered from human gastroenteritis in Tehran, Iran. ournal of global antimicrobial resistance 12:220-226.

Galdeano CM, Cazorla SI, Dumit JML, Vélez E, Perdigón G (2019). Beneficial effects of probiotic consumption on the immune system. Annals of Nutrition and Metabolism 74(2):115-124.

Gomez-Gallego C, Garcia-Mantrana I, Salminen S, Carmen CM (2016). The human milk microbiome and factors influencing its composition and activity. Seminars in Fetal and Neonatal Medicine 21(6):400-405.

Gronlund M, Arvilommi H, Kero P, Lehtonen O, Isolauri E (2000). Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: a prospective follow up study of healthy infants aged 0–6 months. Archives of Disease in Childhood-Fetal and Neonatal Edition 83(3):F186-F192.

Hedberg M, Hasslöf P, Sjöström I, Twetman S, Stecksén-Blicks C (2008). Sugar fermentation in probiotic bacteria–an in vitro study. Oral microbiology and immunology 23(6):482-485.

Jiang M, Zhang F, Wan C, Xiong Y, Shah NP, Wei H, Tao X (2016). Evaluation of probiotic properties of *Lactobacillus plantarum* WLPL04 isolated from human breast milk. Journal of Dairy Science 99(3):1736-1746.

Jomehzadeh N, Javaherizadeh H, Amin M, Saki M, Al-Ouqaili MTS, Hamidi H, Seyedmahmoudi M, Gorjian Z (2020). Isolation and identification of potential probiotic *Lactobacillus* species from feces of infants in southwest Iran. International Journal of Infectious Diseases 96:524-530.

Jost T, Lacroix C, Braegger C, Chassard C (2013). Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. British Journal of Nutrition 110(7):1253-1262.

Kumar H, du Toit E, Kulkarni A, Aakko J, Linderborg KM, Zhang Y, Nicol MP, Isolauri E, Yang B, Collado MC, Salminen S (2016). Distinct patterns in human milk microbiota and fatty acid profiles across specific geographic locations. Frontiers in microbiology

7:1619.

Kurkutia DK, Mistry N, Dwivedi M (2019). Probiotic properties and in vitro biosafety assessment of human Breast milk isolates. Journal of Pure and Applied Microbiology 13(2):1121-1134.

Lashani E, Davoodabadi A, Soltan DMM (2018). Antimicrobial Effects of *Lactobacillus Plantarum and Lactobacillus Paracasei* isolated from honey against Staphylococcus aureus. Journal of Babol University of Medical Sciences 20(3):44-49.

Li SW, Watanabe K, Hsu CC, Chao SH, Yang ZH, Lin YJ, Chen CC, Cao YM, Huang HC, Chang CH, Tsai YC (2017). Bacterial composition and diversity in breast milk samples from mothers living in Taiwan and Mainland China. Frontiers in microbiology 8:965.

Lindgren SE, Dobrogosz WJ (1990). Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS microbiology reviews 7(1-2):149-163.

Marroki A, Zúñiga M, Kihal M, Pérez- Martínez G (2011). Characterization of Lactobacillus from Algerian Goat'S Milk Based on Phenotypic, 16S rDNA Sequencing and their Technological Properties. Brazilian Journal of Microbiology 42(1):158-171.

Martín R, Olivares M, Marín ML, Fernández L, Xaus J, Rodríguez JM (2005). Probiotic potential of 3 Lactobacilli strains isolated from breast milk. Journal of Human Lactation 21(1):8-17.

Memariani M, Najar PS, Mostafavi SKS, Zahraei ST (2014). Detection of class 1 and 2 integrons among enteropathogenic Escherichia coli isolates Archives of Pediatric Infectious Diseases 2(4):e16372.

Navarro-Tapia E, Sebastiani G, Sailer S, Toledano LA, Serra-Delgado M, García-Algar O, Andreu-Fernández V (2020). Probiotic supplementation during the perinatal and infant period: effects on gut dysbiosis and disease. Nutrients 12(8):2243.

Olivares M, Díaz-Ropero MP, Gómez N, Lara-Villoslada F, Sierra S, Maldonado JA, Martín R, López-Huertas E, Rodríguez JM, Xaus J (2006). Oral administration of two probiotic strains, *Lactobacillus gasseri* CECT5714 and *Lactobacillus coryniformis* CECT5711, enhances the intestinal function of healthy adults. International journal of food microbiology 107(2):104-111.

Rammelsberg M, Radler F (1990). Antibacterial polypeptides of *Lactobacillus* species. Journal of Applied Bacteriology 69(2):177-184.

Ruiz L, García-Carral C, Rodriguez JM (2019). Unfolding the human milk microbiome landscape in the omics era. Frontiers in microbiology 10:1378.

Sanders ME, Akkermans LMA, Haller D, Hammerman C, Heimbach J, Hörmannsperger G, Huys G, Levy DD, Lutgendorff F, Mack D, Phothirath P, Solano-Aguilar G, Vaughan E (2010). Safety assessment of probiotics for human use. Gut Microbes 1(3):164-185.

Sharifi Yazdi MK, Davoodabadi A, Khesht Zarrin HR, Tajabadi Ebrahimi M, Soltan Dallal MM (2017). Characterisation and probiotic potential of lactic acid bacteria isolated from Iranian traditional yogurts. Italian Journal of Animal Science 16(2):185-188.

Soccol CR, de Souza VLP, Spier MR, Medeiros ABP, Yamaguishi CT, Lindner JDD, Pandey A, Thomaz-Soccol V (2010). The potential of probiotics: a review. Food Technology and Biotechnology 48(4):413-434.

Soltan Dallal MM, Keshtvarz M, Zamani S, Shirazi M (2016a). Evaluation of anti-microbial activity of *Lactobacillus acidophillus* and *Lactobacillus ruteri* against entero-pathogens by in vitro and in vivo methods. Journal of Gorgan University of Medical Sciences 18(1):45-52.

Soltan Dallal MM, Hosseini M, Davoodabadi A, Rajabi Z, Zamaniahari S (2016b). Isolation and identification of lactic acid bacteria in traditional pickles and salted pickles from Tehran, Iran. Razi Journal of Medical Sciences 23(143):81-90.

Soltan Dallal MM, Khesht Zarrin HR, Tajabadi Ebrahimi M, Davoodabadi A, Hakimian MM, Sadrabadi AA, Sharifi Yazdi MK (2016c)

.

Soltan Dallal MM, Zamaniahari S, Davoodabadi A, Hosseini M, Rajabi Z (2017a). Identification and characterization of probiotic lactic acid bacteria isolated from traditional persian pickled vegetables. GMS hygiene and infection control 12:1-7.

Soltan Dallal MM, Davoodabadi A, Abdi M, Hajiabdolbaghi M, Sharifi Yazdi MK, Douraghi M, Tabatabaei Bafghi SM (2017b). Inhibitory effect of *Lactobacillus plantarum* and Lb. *fermentum* isolated from the faeces of healthy infants against nonfermentative bacteria causing nosocomial infections. New Microbe new infections15:9-13.

Soto A, Martín V, Jiménez E, Mader I, Rodríguez JM, Fernández L (2014). Lactobacilli and bifidobacteria in human breast milk: influence of antibiotherapy and other host and clinical factors. Journal of pediatric gastroenterology and nutrition 59(1):78-88.

Taghizadeh M, Ghasemian SH, Poursina F (2017). Identification of *Lactobacillus plantarum* in Breast Milk. Research in Molecular Medicine (RMM) 5(4):50-60.

Talebreza A, Memariani M, Memariani H, Shirazi MH, Shamsabad PE, Bakhtiari M (2016). Prevalence and antibiotic susceptibility of Shigella species isolated from pediatric patients in Tehran. Archives of Pediatric Infectious Diseases 4(1).

Urbaniak C, Angelini M, Gloor GB, Reid G (2016). Human milk microbiota profiles in relation to birthing method, gestation and infant gender. Microbiome 4(1):1-9.

Wadher KJ, Mahore JG, Umekar MJ (2010). Probiotics: living medicines in health maintenance and disease prevention. International Journal of pharma and bio sciences 1(3):1-9.

Xanthopoulos V, Ztaliou I, Gaier W, Tzanetakis N, Litopoulou-Tzanetaki E (1999). Differentiation of Lactobacillus isolates from infant faeces by SDS-PAGE and rRNA-targeted oligonucleotide probes. Journal of applied microbiology 87(5):743-749.

Yuki N, Watanabe K, Mike A, Tagami Y, Tanaka R, Ohwaki M, Morotomi M (1999). Survival of a probiotic, *Lactobacillus casei* strain Shirota, in the gastrointestinal tract: selective isolation from faeces and identification using monoclonal antibodies. International journal of food microbiology 48(1):51-57.

Zimmermann P, Curtis N (2020). Breast milk microbiota: A review of the factors that influence composition. Journal of Infection 81(1):17-47.

Vol. 15(6), pp. 325-333, June, 2021
DOI: 10.5897/AJMR2020.9413
Article Number: C331A3467059
ISSN: 1996-0808
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# Screening for the prevalence of *Helicobacter pylori* infection among dyspeptic patients using simple fecal antigen and serum antibody diagnostic methods at Mukalla city Hospitals, Hadhramout, Yemen

**Eidha Ali Bin-Hameed* and Huda Mohammed Barajash**

Department of Biology, Faculty of Sciences, Hadhramout University, Yemen.

Received 26 August, 2020; Accepted 25 May, 2021

***Helicobacter pylori*** **is the most common infection in the world, and the most main causes of dyspepsia are related to *H. pylori* infection. In that respect, several non-invasive methods for the diagnosis of *H. pylori* infection were utilized. The current study aimed to determine the prevalence of *H. pylori* infection and its associated variables among dyspeptic patients in Mukalla city, Hadhramout-Yemen during a period from February to September 2018. A cross-sectional descriptive study was conducted among 100 suspected dyspeptic patients. Data regarding to associated variables were collected using a structured questionnaire, and the samples of feces and serum were collected and screened for *H. pylori* by rapid diagnostic immunochromatographic assays for antigen and antibody. Data was analyzed using SPSS statistical software version 20 and a *P* value <0.05 was taken statistically significant. Prevalence of *H. pylori* was found to be 15 and 18.5% using the antigen *H. pylori* test and serum anti-*H. pylori* test respectively. Prevalence using antigen *H. pylori* test was significantly associated to male sex (P=0.035). In those patients with *H. pylori*, a positive result with antigen test was significantly associated to drinking non-filtered water (COR = 3.67; 95%CI=1.436-9.363; P=0.007), symptoms of heartburn and regurgitation (COR=0.865, 95%CI=0.034-0.536, P=0.004) and antibiotics used (COR=0.312, 95%CI=0.125-0.780, P=0.013). The prevalence of *H. pylori* infection was frequent among the dyspeptic patients in the study area. *H. pylori* infection was related to non-filtered water source, symptoms of heartburn and regurgitation and antibiotics used are contributing factors. Moreover, further studies are needed to investigate other potential associated variables for *H. pylori* infection.**

**Key words:** Antibody, antigen, dyspepsia, *Helicobacter pylori*, laboratory diagnosis, prevalence.

## INTRODUCTION

*Helicobacter pylori* infects about 30-50% of the general population worldwide and its infection acquired during early childhood (Liu et al., 2017). *H. pylori* is recognized to play a causative role in the pathogenesis of various gastrointestinal diseases including peptic ulcer, chronic gastritis and it has been etiologically associated with

*Corresponding author. E-mail: eidha6@gmail.com. Tel: +967 773568330.

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mucosa-associated lymphoid tissue (MALT) lymphoma and gastric carcinoma (Chandrababu et al., 2016; Nevoa et al., 2017).

Dyspepsia is a common health problem worldwide with an overall prevalence 25%. The diagnosis and management of dyspepsia causes a lot of economic burden worldwide (Naz et al., 20013). Dyspepsia symptoms often include epigastric pain, postprandial fullness, and/or early satiety. In dyspeptic patients without infection of *H. pylori*, it has been shown that ulcer disease is very rare and endoscopic examination is usually normal or shows evidence of oesophagitis (Wong and McLean, 2016). Dyspepsia also is a common presenting complaint of various upper gastrointestinal disorders. There are many causes of dyspepsia and the major ones include peptic ulcer disease (PUD), gastroduodenitis, esophagitis, malignancies, parasitic infection and dyspepsia (Ayana et al., 2014).

Although the route of transmission of *H. pylori* infection is not clearly known, some evidences indicate the main route is by contaminated water and food and faecal-oral transmission (Shiferaw and Abera, 2019), but it is not clear if specific foods can increase the risk of *H. pylori* infection or not (AlKalbani et al., 2016). *H. pylori* infection is apparent significantly higher in the developing countries than in the developed countries and the distribution varies between geographical locations and different communities (Lu et al., 2018). Most people get their primary infection of *H. pylori* during the childhood stage and stay infected during their life time (Atherton and Blaser, 2009). Also, there is no agreeable on the state of being male or female as a risk factor for *H. pylori* infection, but the physiological differences between male and female may affect the mechanism of immune response to the pathogenesis of bacteria (Ibrahim et al., 2017).

Non-invasive diagnostic tests for detection the antigen and antibody directed against *H. pylori* are now available and these tests are valuable both for screening and evaluation of therapy (Lee et al., 2013), and these non-invasive tests are also easy and cheap to perform (Formichella et al., 2013). The advantage of antigen detection test is to evaluate the eradication of *H. pylori* infection. However, if antigen concentration becomes low, false negative results may be reported. Fecal antigen detection *H. pylori* has certain disadvantages like antigen excretion may vary over the time period and may degrade while passing through the intestine (Patel et al., 2014).

In countries with low socioeconomic status like Yemen, there is poor personal hygienic practice, poor waste disposal system, crowded living conditions and lack of clean and safe water which are known to make individuals susceptible for *H. pylori* infection. Currently, large groups of populations predisposing to several risk factors of *H. pylori* infection. So, the prevalence and the epidemiological patterns of *H. pylori* infection varies greatly among societies and geographical locations. On a wider level, there is continuing need for numerical data on *H. pylori* infection distribution, its determinant and potential risk factors associated it in order to identify priorities for the health services in the community. On the other hands, studies on prevalence of *H. pylori* infection are important to design the appropriate interventions strategies. Therefore, the current study was aimed to evaluating the potential associated variables with the prevalence of *H. pylori* infection among dyspeptic patients by using fecal antigen and serum antibody diagnostic methods in Mukalla city hospitals, Hadhramout Governorate, Yemen.

## PATIENTS AND METHODS

### Study design and study population

This cross-sectional descriptive study was conducted among suspected cases of patients complaining of dyspepsia referred to some main hospitals in Mukalla city, Hadhramout governorate, Yemen during a period from February to September 2018.

### Inclusions criteria

The patients showing symptoms of dyspeptic disorders were included in this study. Dyspepsia was defined as the presence of 2 or more of the following symptoms; heartburn, acid regurgitation, increased abdominal bloating, nausea, feeling of abnormal or slow digestion, or early satiety (Duvnjak, 2011).

### Sample size calculation

The following formula was used for the sample size calculation (Samson et al., 2018):

$$n = (1.96)^2 pq / d^2$$

Where:

n = required sample size
p = proportion of the population having *H. pylori* infection from previous study
q = 1 - p
d = the degree of precision

For the calculation, a 95% confidence interval, P of 0.375, that is, a prevalence rate of 37.5% from previous study by Moharram et al. (2015) and margin of error (d) set at 0.05 was used to determine the minimum sample size required. To minimize errors arising from the likelihood of non-compliance, 15% of the sample size was added to obtain the final sample size. Therefore, a convenience sample of 100 suspected dyspeptic patients were included in the study and examined for *H. pylori* fecal antigen and anti-*H. pylori* serum rapid tests.

### Data collection tool

A standardized, interviewer-administered, structured questionnaire was developed to obtain data regarding *H. pylori* infection associated variables. It consisted of systematic questions on demographic variables of gender, age groups, educational level,

monthly income level and family size; personal habits and hygienic behavior variables of food patterns, water sources and smoking; clinical variables of symptoms associated with infection and antibiotics drug used. The questionnaire filled with the aid of an interviewer.

### Laboratory analysis

One step *H. pylori* antigen test is a rapid, serological, immunochromatographic assay supplied by InTec Products, INC, USA for detection of *H. pylori* antigen in human feces sample. The test is used to obtain a visual, qualitative result with a high sensitivity 98.4% and specificity 98.6% as per manufacturer's specifications. When feces sample is added to sample pad, it moves through the conjugate pad and mobilizes gold anti-*H. pylori* conjugate that is coated on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with anti-*H. pylori* that is coated on the test region. If *H. pylori* is present, the result is the formation of a colored band in the test region. If there is no *H. pylori* in the sample the area will remain colorless. The sample continues to move to the control area and forms a pink color which indicating the test is working and the result is valid.

Serum anti-*H. pylori* was detected using a one-step anti-*H. pylori* test cassette supplied by InTec Products, INC, USA. The advanced quality rapid anti-*H. pylori* test is a colloidal gold enhanced test detect antibodies in human. The test is based on immunochromatography and can give a result within 20 min with a high sensitivity of 94.88% and specificity of 95.21% as per manufacturer's specifications. The test is used for the rapid visual qualitative detection of antibodies to *H. pylori* in human serum, plasma or whole blood. The procedures followed the manufacturer's instructions. In brief, 100 µl of sample or control was dispensed into the circular sample well on the card, then the test results interpreted at 15-20 min.

### Ethics approval and consent to participate

Research ethical approval of this study was obtained from Hadhramout University, Faculty of Sciences. Written consent was obtained, meeting No. HU/FOS/P1/11/2017, date 12/11/2017 before commencing the study. Permission letter was obtained from the hospital's administrations. The information was taken from the participants after they agreed to it verbally according to the informed consent with confidentiality of each study participant's result.

### Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20 was used for data analysis. The association between different variables and outcome of *H. pylori* infection was calculated and compared using Pearson Chi-square ($\chi 2$) test. Binary and multiple regression tests (crude odds ratio/adjusted odds ratio) were used to detect independent predictors of *H. pylori* positivity in dyspeptic patients. The level of statistical significance was set at p-value < 0.05.

## RESULTS

### Socio-demographic characteristics of study participants

A total of 100 suspected dyspeptic patients participated in this study. Majority of the study participants were females 53%. The mean age of participants was 25.7±15.0 SD (age range 5-60 years old). Regarding to educational status, primary and high school accounted for the highest percentage, 35 and 26% respectively. About 83% of the participants had a medium monthly income. Family size of 6-8 persons was the highest percentage 44% as demonstrated in Table 1.

### Prevalence of *H. pylori* infection and associated variables

Prevalence of *H. pylori* was found to be 15 and 18.5% using the antigen *H. pylori* test and serum anti-*H. pylori* test respectively. The highest prevalence of *H. pylori* infection was seen among the males than the females 63% vs 37% by antigen test with the difference was statistically significant (COR=0.386, 95%CI=0.160-0.934, *P*=0.035), and 57% vs 43% by antibody test, but the difference was not statistically significant (*P*=0.136). The highest percentage, 40.0% of *H. pylori* infections was noticed in the age group of 19-32 years old participants, followed by the age group of 33-46 years old 33.0% for antigen test, whereas the highest percentage, 38.0% of *H. pylori* infections showed in the age group of 33-46 years old participants, followed by the age group of 19-32 years old 35.0% for antibody test with no difference significant associated. Regarding the educational status, the primary and high school levels are the majority groups who come up positive for *H. pylori* both in the antigen and antibody tests than others with no statistically significant difference (*P* > 0.05). Prevalence of *H. pylori* infection was higher among medium income level participants, and number of family members of 6-8 and more than 9 persons showed high infection with *H. pylori* both in the antigen and antibody tests with no statistically significant difference (*P* > 0.05). In those patients with *H. pylori,* a positive result with antigen test, non-filtered water source had 3.667 times risk of getting *H. pylori* infection (95%CI=1.436-9.363, *P*=0.007) and not significant for positive result of antibody test. Also, food pattern and smoking were not associated with *H. pylori* infection (*P* > 0.05) for both positive results in the antigen and antibody tests. Clinically, the patients with heartburn, heartburn and regurgitation had come up with positive for the *H. pylori* antigen and antibody tests, and likewise, heartburn and regurgitation are significantly associated (COR=0.865, 95%CI=0.034-0.536, *P*=0.004) for antigen test, and antibody test (COR=0.676, 95%CI=0.103-1.015, *P*=0.053). Patients with *H. pylori* positive result with antigen test, antibiotics used is significant associated (COR=0.312, 95%CI=0.125-0.780, *P*=0.013), but not significant with a positive result with antibody test (*P*=0.138) as shown in Tables 2 and 3.

Multivariate logistic regression analysis was performed for these associated variables that showed significant at the crude odds ratio calculation for fecal antigen test. The association remains significant between *H. pylori*

**Table 1.** Socio-demographic characteristics of suspected dyspeptic patients attending the hospitals at Mukalla city, Hadhramout, Yemen.

| Variable | | Frequency | Percentage |
|---|---|---|---|
| Gender | Male | 47 | 47 |
| | Female | 53 | 53 |
| Age group (years) | 5 - 18 | 3 | 3 |
| | 19 - 32 | 38 | 38 |
| | 33 - 46 | 37 | 37 |
| | 47 - 60 | 22 | 22 |
| Educational level | Primary | 35 | 35 |
| | High school | 26 | 26 |
| | University | 16 | 16 |
| | Postgraduate | 1 | 1 |
| | Illiterate | 22 | 22 |
| Income level | High | 1 | 1 |
| | Medium | 83 | 83 |
| | Low | 16 | 16 |
| Family size (person) | 3 - 5 | 28 | 28 |
| | 6 - 8 | 44 | 44 |
| | 9 and more | 28 | 28 |

**Table 2.** Fecoprevalence of *H. pylori* infection and its associated variables among the dyspeptic patients.

| Variable | | No. of cases | Positive fecal Ag(%) | COR | CI(95%) | p-value |
|---|---|---|---|---|---|---|
| Gender | Male | 47 | 19(63.0) | 0.386 | 0.160-0.934 | 0.035* |
| | Female | 53 | 11(37.0) | 1 | 1 | |
| Age group (years) | 5 - 18 | 3 | 1(3.0) | 0.067 | 0.072-12.105 | 0.958 |
| | 19 - 32 | 38 | 12(40.0) | 1.011 | 0.327-3.124 | 0.985 |
| | 33 - 46 | 37 | 10(33.0) | 1.260 | 0.397-3.995 | 0.695 |
| | 47 - 60 | 22 | 7(24.0) | 1 | 1 | |
| Educational level | Primary | 35 | 6(20.0) | 1.813 | 0.501-6.556 | 0.365 |
| | High school | 26 | 10(33.0) | 0.4 | 0.176-2.046 | 0.414 |
| | University | 16 | 7(24.0) | 0.518 | 0.123-1.883 | 0.294 |
| | Postgraduate | 1 | 1(3.0) | 0.000 | 0.000 | 1.000 |
| | Illiterate | 22 | 6(20.0) | 1 | | |
| Income level | High | 1 | 0(0.0) | 372801891.8 | 0.000 | 1.000 |
| | Medium | 83 | 27(90.0) | 0.521 | 0.126-1.822 | 0.280 |
| | Low | 16 | 3(10.0) | 1 | | |
| Family size (person) | 3 - 5 | 28 | 6(20.0) | 2.037 | 0.621-6.686 | 0.241 |
| | 6 - 8 | 44 | 14(47.0) | 1.190 | 0.438-3.236 | 0.733 |
| | 9 and more | 28 | 10(33.0) | 1 | | |
| Food pattern | Fatty, citrus and spicy | 93 | 27(90.0) | 1.833 | 0.384-.8.746 | 0.447 |
| | Nothing | 7 | 3(10.0) | 1 | | |

**Table 2.** Cont'd.

| | | | | | | |
|---|---|---|---|---|---|---|
| Water source | Non filtered | 48 | 8(27.0) | 3.667 | 1.436-9.363 | 0.007* |
| | Filtered | 52 | 22(73.0) | 1 | | |
| Behavior | Smoking | 11 | 4(13.0) | 0.278 | 0.195-2.678 | 0.627 |
| | Nothing | 89 | 26(87.0) | 1 | | |
| Clinical symptoms | Regurgitation | 4 | 0(0.0) | 269245810.7 | 0.000 | 0.999 |
| | Heartburn | 37 | 6(20.0) | 0.139 | 0.258-5.219 | 0.845 |
| | Heartburn and regurgitation | 38 | 21(70) | 0.865 | 0.034-0.536 | 0.004* |
| | No symptoms | 21 | 3(10.0) | 1 | | |
| Antibiotics used | Used | 29 | 14(47.0) | 0.312 | 0.125-0.780 | 0.013* |
| | Non-used | 71 | 16(53.0) | 1 | | |

*Significant statistics at p-value <0.05; COR, Crude Odds Ratio; CI, confidence interval.

infection, gender, non-filtered water source, symptoms of heartburn and regurgitation and antibiotics used as given in Table 4.

## DISCUSSION

*H. pylori* is a common health problem worldwide and its infection is an important public health in developed and developing countries (Ozbey and Hanafiah, 2017). Here in Hadhramout, Yemen to the best of our knowledge, no data exist on the prevalence of *H. pylori* infection and associated risk factors. Therefore, the present study was designed to determine the prevalence of *H. pylori* infection and associated variables among dyspeptic patients in Mukalla city, Hadhramout governorate. In this study, the overall feco and seroprevalence of *H. pylori* infection was 15 and 18.5% which was similar to studies results of fecoprevalence *H. pylori* done in Iran 37.8% (Iranikhah et al., 2013), Nigeria 23.5% (Samson et al., 2018), Ethiopia 36.8% (Shiferaw and Abera, 2019). Similar results of seroprevalence *H. pylori* showed in Vietnam 48.8% (Nguyen et al., 2017), Nigeria 28.0 and 36.3% (Samson et al., 2018; Daniyan et al., 2020). However, the results obtained in this study is lower than those of previous studies of anti-*H. pylori* seropositivity 98% (Misganaw and Abera, 2017), 64.39% (Mabeku et al., 2018), 51.4% (Chukwuma et al., 2020), 73.11 and 58.05% for dyspeptic diabetic patients and non-diabetic respectively (Mabeku et al., 2020), and fecoprevalence 81.8% (Sabh and El-Less, 2017). The reason for this variation could be due to difference in sample size, diagnostic methods, sanitation practice level of individuals and communities, living conditions or low levels of exposure to the risk factors other than that included in our study. Moreover, some of the above studies used anti-*H. pylori* testing which may lead to over estimation of the infection prevalence.

In the current study, the proportion of *H. pylori* was higher in males than females, and the difference was statistically significant. Although the number of females was greater than males participants; this might be the reason why the prevalence of *H. pylori* in male participants was higher than females. We need further researches on gender specific *H. pylori* infection to explain the variation. This finding was consistent with previous studies (Broutet et al., 2001; Chandrababu et al., 2016; Ibrahim et al., 2017). In contrast, other studies showed that *H. pylori* infection was significantly higher in females than males (Samson et al., 2018; Mabeku et al., 2018). While some studies showed no relation observed between the gender and *H. pylori* infection in dyspeptic patients (Roland et al., 2016; Dilnessa and Amentie, 2017; Tameshkel et al., 2018).

Our study indicated high proportion of *H. pylori* infection in the age groups of 19-32 years and 33-46 years with insignificant difference. This was comparable to different previous studies (Zhu et al., 2014; Chandrababu et al., 2016; Mabeku et al., 2018; Alharbi and Ghoraba, 2019). Contrary to this, other studies reported the average age of infected patients of *H. pylori* in the age group of 40-49 years (Simón et al., 2016) and 50–59 years (Syam et al., 2015), other studies showed a predictor of *H. pylori* infection takes place early in childhood (Mabeku et al., 2018; Daniyan et al., 2020). Other studies showed no relation observed between age groups with *H. pylori* infection (Roland et al., 2016; Dilnessa and Amentie, 2017), while other studies revealed a statistically significant association between *H. pylori* positivity and age of patients (Moharram et al., 2015; Tameshkel et al., 2018).

In this study, primary and high school education level of participants accounts the majority positive for *H. pylori* with no significant association, and this comparable with

**Table 3.** Seroprevalence of *H. pylori* infection and its associated variables among the dyspeptic patients.

| Variable | | No. of cases | Positive serum Ab(%) | COR | CI(95%) | p-value |
|---|---|---|---|---|---|---|
| Gender | Male | 47 | 21(57.0) | 0.535 | 0.235-1.217 | 0.136 |
| | Female | 53 | 16(43.0) | 1 | | |
| Age group (years) | 5 - 18 | 3 | 2(5.0) | 0.714 | 0.022-3.669 | 0.336 |
| | 19 - 32 | 38 | 13(35.0) | 1.099 | 0.367-3.292 | 0.866 |
| | 33 - 46 | 37 | 14(38.0) | 0.061 | 0.314-2.802 | 0.910 |
| | 47 - 60 | 22 | 8(22.0) | 1 | | |
| Educational level | Primary | 35 | 12(32.0) | 1.327 | 0.442-3.985 | 0.614 |
| | High school | 26 | 7(19.0) | 1.879 | 0.558-6.326 | 0.308 |
| | University | 16 | 8(22.0) | 0.308 | 0.189-2.533 | 0.578 |
| | Postgraduate | 1 | 1(3.0) | 0.000 | 0.000 | 1.000 |
| | Illiterate | 22 | 9(24.0) | 1 | | |
| Income level | High | 1 | 0(0.0) | 1615474866 | 0.000 | 1.000 |
| | Medium | 83 | 29(78.0) | 1.862 | 0.633-5.477 | 0.259 |
| | Low | 16 | 8(22.0) | 1 | | |
| Family size (person) | 3 - 5 | 28 | 9(24.0) | 2.111 | 0.713-6.249 | 0.177 |
| | 6 - 8 | 44 | 14(38.0) | 2.143 | 0.808-5.683 | 0.126 |
| | 9 and more | 28 | 14(38.0) | 1 | | |
| Food pattern | Fatty, citrus and spicy | 93 | 33(89.2) | 2.424 | 0.511-11.491 | 0.265 |
| | Nothing | 7 | 4(10.8) | 1 | | |
| Water source | Non filtered | 48 | 15(41.0) | 1.613 | 0.709-3.669 | 0.254 |
| | Filtered | 52 | 22(59.0) | 1 | | |
| Behavior | Smoking | 11 | 5(13.5) | 0.674 | 0.190-2.383 | 0.540 |
| | Nothing | 89 | 32(86.5) | 1 | | |
| Clinical symptoms | Regurgitation | 4 | 0(0.0) | 646189945.7 | 0.000 | 0.999 |
| | Heartburn | 37 | 10(27.0) | 1.080 | 0.328-3.560 | 0.899 |
| | Heartburn and regurgitation | 38 | 21(56.8) | 0.676 | 0.103-1.015 | 0.053* |
| | No symptoms | 21 | 6(16.2) | 1 | | |
| Antibiotics used | Used | 29 | 14(38.0) | 0.513 | 0.213-1.240 | 0.138 |
| | Non-used | 71 | 23(62.0) | 1 | | |

*Significant statistics at p-value <0.05; COR, Crude Odds Ratio; CI, confidence interval

some studies showed low and high school educational level was more infected with *H. pylori* infection (Dilnessa and Amentie, 2017; Shiferaw and Abera, 2019), but the predictor of seroprevalence of *H. pylori* infection was found to be the illiteracy (Hamrah et al., 2017). This result could be due to the fact that low education level has a significant impact on personal and environmental hygiene and play a role in the prevalence of *H. pylori* infection.

In our finding, *H. pylori* infection was higher in medium–income households, which was incomparable with other reports that have identified low-income as a risk factor predisposing to *H. pylori* infection (Subsomwong et al., 2017; Mabeku et al., 2018; Shiferaw and Abera, 2019). Other studies revealed that the crowded household was a predictive factor for *H. pylori* infection (Nguyen et al., 2017; Bello et al., 2018; Gide et al., 2019).

In addition to fecal-oral transmission of *H. pylori*, source of drinking water has been noticed as one of the contributing risk factors for the high prevalence of *H. pylori* infection in developing countries, where there is a

**Table 4.** Adjusted odds ratio for gender, water source, clinical symptoms and antibiotics used with *H. pylori* infection among dyspeptic patients.

| Variable | | Fecal antigen test | | |
|---|---|---|---|---|
| | | AOR | CI(95%) | p-value |
| Gender | Male | 2.591 | 1.071-6.267 | 0.035* |
| | Female | 1 | 1 | |
| Water source | Non filtered | 0.727 | 0.107-0.696 | 0.007* |
| | Filtered | 1 | 1 | |
| Clinical symptoms | Regurgitation | - | - | - |
| | Heartburn | 0.161 | 0.258-5.219 | 0.845 |
| | Heartburn and regurgitation | 7.412 | 1.866-29.444 | 0.004* |
| | No symptoms | 1 | 1 | |
| Antibiotics used | Used | 3.208 | 1.283-8.024 | 0.013* |
| | Non-used | 1 | 1 | |

*Significant statistics at p-value <0.05; AOR Adjusted odds ratio; CI, confidence interval

lack of access to clean water and poor sewerage system (Nurgalieva et al., 2002). The relationship between source of drinking water and *H. pylori* infection was statistically significant in this study. This result was similar to other previous studies (Subsomwong et al., 2017; Bello et al., 2018; Shiferaw and Abera, 2019; Chukwuma et al., 2020).

Individuals who regularly eat uncooked vegetables are more likely to be infected with *H. pylori* (Cover and Blaser, 2009). From this study, we investigated a particular type of food (that is, the spicy, citrus or fatty foods) was positively associated with *H. pylori* infection with no statistically significant difference. Some changes in the gastric mucosa may be associated with an increased chance of persistent infection with *H. pylori* such as the way in which food is prepared, and dietary administration of salt may induce mucosal damage and destroy the mucosal barrier in the stomach (Zhu et al., 2014). Some previous studies showed the prevalence of *H. pylori* infection was associated with the consumption of a particular type of food, in Yemen fat rich meals (Moharram et al., 2015), in Ethiopia coffee consumption and alcohol drinking (Dilnessa and Amentie, 2017), in Pakistan junk food and beverages (Gul et al., 2016), in China eating kipper and fried food (Zhu et al., 2014). A study conducted in Yemen revealed statistically significant association between *H. pylori* positivity with Qat chewing (Moharram et al., 2015), other study carried out in Ethiopia showed Qat chewing had no significant association with *H. pylori* infection (Dilnessa and Amentie, 2017). Other findings found agree with our results with no statistically significant association between *H. pylori* positivity and smoking (Zhu et al., 2014; Moharram et al., 2015; Dilnessa and Amentie, 2017), while other study showed a cigarette smoking was significant risk factor for infection of *H. pylori* (Bello et al., 2018).

In our results, the relationship of *H. pylori* infection prevalence with clinical symptoms of dyspepsia like heartburn and regurgitation were statistically significant associated. The data were confirmed by multivariate logistic regression analysis. Similar results of other studies revealed that *H. pylori* infection was associated with dyspepsia (Misganaw and Abera, 2017; Tameshkel et al., 2018; Borges et al., 2019; Mabeku et al., 2020). Other study showed the predictor of seroprevalence *H. pylori* was found to be epigastric pain (Hamrah et al., 2017). However, *H. pylori* is motile even in the highly viscous mucus layer, and it can evade gastric motility, peristalsis and gastric acidity. Although it is motile, it may adhere to the gastric mucosa through specific adhesion mechanisms, and secretion of large amounts of virulence factors such as urease, cytotoxins, proteases and phospholipases results in in the intense acidity of the stomach, cause local inflammation, attack and damage mucosal cell membranes (Shi et al., 2008). In some cases, *H. pylori* can be detected only in the stomach, particularly during proton pump inhibitors (PPIs) use (Wong and McLean, 2016). Antibiotics drug usage was statistically significant associated with *H. pylori* infection in the current study. Other agreement results showed that the antibiotics used was found to be significant risk factor for *H. pylori* infection (Moharram et al., 2015; Nguyen et al., 2017). Antibiotics resistance in *H. pylori* is an increasing trend because of the overuse and misuse worldwide of antibiotics for the treatment of other infections, especially in developing countries (Mégraud, 2013), and this is resulting in falling success rates of *H.*

*pylori* eradication treatment (Suzuki, 2019).

## Limitations

The limitation of this study was the lack of a control (non-dyspeptic) group. The sample size of the study is relatively small. For diagnostic test involving sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy all these deal with how efficient a test must use the gold standard technique. Lack of advancing techniques such as invasive or molecular diagnostic methods. Using a cross-sectional descriptive study design impeding the determination of causality, therefore we anticipate that future studies with a longitudinal or survey study design over more extended periods of time.

## Conclusion

The study revealed that the prevalence of *H. pylori* infection was frequent among the dyspeptic patients. Highly prevalence of *H. pylori* infection was found among males, the age groups 19-32 years and 33-46 years, primary and high school educational levels, medium monthly income level and overcrowded house. The prevalence of *H. pylori* infection was found significantly associated with gender. Also, heartburn and regurgitation, sources of drinking water and antibiotics drug used were statistically significant associated variables with *H. pylori* infection, while there was no statistically significant association between *H. pylori* positivity and type of foods and smoking. Non-invasive *H. pylori* testing cards was rapid, easy, inexpensive methods for detection *H. pylori* and important for screening the infection and epidemiological studies. Further longitudinal studies must do in depth using different diagnostic testing methods.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

This work was supported by the Biology Department, Faculty of Science, Hadhramout University, Ibn-Sina Teaching Hospital Lab, and Bawazeer Private Medical Labs for medical analysis at Mukalla City, Hadhramout Governorate, Yemen.

### REFERENCES

Alharbi RH, Ghoraba M (2019). Prevalence and patient characteristics of *Helicobacter pylori* among adult in primary health care of security forces hospital Riyadh, Saudi Arabia, 2018. Journal of Family Medicine and Primary Care 8:2202-2206.

AlKalbani SRA, Al-Hinai FTNAM, AlMuniri A (2016). Diet and lifestyle factors and the risk of *H. pylori* infection in Omani patients attending SQUH Daycare for OGD. Journal Family Medicine Community Health 3(2):1077.

Atherton JC, Blaser MJ (2009). Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications. Journal of Clinical Investigations 119(9):2475-287.

Ayana SM, Swai B, Maro VP, Kibiki GS (2014). Upper gastrointestinal endoscopic findings and prevalence of *Helicobacter pylori* infection among adult patients with dyspepsia in northern Tanzania. Tanzania Journal of Health Research 16(1):1-9.

Bello AK, Umar AB, Borodo MM (2018). Prevalence and risk factors for *Helicobacter pylori* infection in gastroduodenal diseases in Kano, Nigeria. African Journal of Medical and Health Sciences 17:41-61.

Borges SS, Ramos AFPL, Filho M, Cadsb B, Carneiro LC, Barbosa MS (2019). Prevalence of *Helicobacter pylori* infection in dyspeptic patients and its association with clinical risk factors for developing gastric adenocarcinoma. Arquivos de Gastroenterologia pii S0004-28032019005001103.

Broutet N, Sarasqueta AM, Sakarovitch C, Cantet F, Lethuaire D, Mégraud F (2001). *Helicobacter pylori* infection in patients consulting gastroenterologists in France: prevalence is linked to gender and region of residence. European Journal of Gastroenterology and Hepatology 13(6):677-684.

Chandrababu D, Nandeesh HP, Suvarna D, Aradya HV, Vijaykumar TR, Suresh I, Kothe SR (2016). Association of *H. pylori* infection with gastroduodenal disease: a cross sectional study from Mysuru, India. National Journal of Medical Research 6(3):292-295.

Chukwuma OM, Chukwuma GO, Manafa PO, Akulue JC, Jeremiah ZA (2020). Prevalence and possible risk factors for *Helicobacter pylori* seropositivity among peptic ulcerative individuals in Nnewi Nigeria. BioMed Research Journal 4(1):166-172.

Cover TL, Blaser MJ (2009). *Helicobacter pylori* in health and disease. Gastroenterology 136(6):1863-1873.

Daniyan OW, Ibe CB, Ezeonu TC, Anyanwu OU, Ezeanosike OB, Omeje KN (2020). Seroprevalence and risk factors of *Helicobacter pylori* infection among children in South-East Nigeria. Journal of Gastroenterology and Hepatology Research 9(1):3066-3070.

Dilnessa T, Amentie M (2017). Prevalence of *Helicobacter pylori* and risk factors among dyspepsia and non-dyspepsia adults at Assosa General Hospital, West Ethiopia: A comparative study. Ethiopian Journal Health Development 31(1):4-12.

Duvnjak M (2011). Dyspepsia in clinical practice. Springer. New York . pp. 4-5.

Formichella L, Romberg L, Bolz C, Vieth M, Geppert M, Göttner G, Nölting C, Walter D, Schepp W, Schneider A, Ulm K, Wolf P, Busch DH, Soutschek E, Gerhard M (2013). A novel line immunoassay based on recombinant virulence factors enables highly specific and sensitive serologic diagnosis of *Helicobacter pylori* infection. Clinical Vaccine and Immunology 20:1703-1710.

Gide S, Ibrahim Y, Anas G, Alegbe SD (2019). Prevalence of *Helicobacter pylori* infection in patients with dyspeptic symptoms in Damaturu Metropolitan. Journal of Advances in Microbiology 18(2):1-8.

Gul S, Jawed L, Tariq S, Aziz S (2016). *Helicobacter pylori* association with acid peptic disease: It's incidence in population having increase junk food intake. Translational Biomedicine 7(4):90.

Hamrah MH, Hamrah MS, Hamrah MH, Kanda M, Hamrah AE, Dahi AE, Homayoun F, Hamrah MH, Fotouhi A, Sakamoto J (2017). Prevalence of *Helicobacter pylori* infection in dyspeptic patients in Andkhoy Afghanistan. Asian Pacific Journal of Cancer Prevention 18(11):3123–3127.

Ibrahim AMS, Ferro A, Lunet N, Peleteiro B (2017). Sex-differences in the prevalence of *Helicobacter pylori* infection in pediatric and adult populations: systematic review and meta-analysis of 244 studies. Digestive and Liver Diseases 49(7):742-749.

Iranikhah A, Ghadir MR, Sarkeshikian S, Saneian H, Heirari A, Mahvari M (2013). Stool antigen tests for the detection of *Helicobacter pylori* in children. Iranian Journal of Pediatrics 23(2):138-142.

Lee HC, Huang CL, Lin KY, Chen KW, Wu DC (2013). Performance of

routine *Helicobacter pylori* invasive tests in patients with dyspepsia. Gastroenterology Research and Practice 184806. DOI: 10.1155/2013/184806.

Liu X, Nie W, Liang J, Li Y (2017). Interaction of *Helicobacter pylori* with other microbiota species in the development of gastric cancer. Archives of Clinical Microbiology 8(2):37. DOI: 10.4172/1989-8436.100067.

Lu C, Yu Y, Li L, Yu C, Xu P (2018). Systematic review of the relationship of *Helicobacter pylori* infection with geographical latitude, average annual temperature and average daily sunshine. BMC Gastroentrology18(1):50.

Mabeku LBK, Ngamga MLN, Leundji H (2018). Potential risk factors and prevalence of *Helicobacter pylori* infection among adult patients with dyspepsia symptoms in Cameroon. BMC Infectious Diseases 18(1):1-11. DOI: 10.1186/s12879-018-3146-1

Mabeku LBK, Noundjeu ML, Leundji NH (2020). *Helicobacter pylori* infection, a risk factor for Type 2 diabetes mellitus: a hospital‑based cross‑sectional study among dyspeptic patients in Douala‑Cameroon. Scientific Reports 10:12141. https://doi.org/10.1038/s41598-020-69208-3

Mégraud F (2013). Current recommendations for *Helicobacter pylori* therapies in a world of evolving resistance. Gut Microbes 4(6):541-548.

Misganaw B, Abera A (2017). The relationship between dyspepsia and *H. pylori* infection in Southern Ethiopia. Journal of Medicine and Medical Sciences 6(5):086-090. ISSN: 2315-5159.

Moharram ASS, Alqady A, Alhetary AA (2015). Prevalence of *Helicobacter pylori* among gastritis patients in Sana'a, Yemen. International Journal of Current Microbiology and Applied Sciences 4(3):769-778.

Naz F, Malik S, Afzal S, Anwar SA (2013). Frequency of seropositivity of *Helicobacter pylori* in patients presenting with dyspepsia. Journal Ayub Medical College Abbottabad 25(3-4):50-54.

Nevoa JC, Rodrigues RL, Menezes GL, Lopes AR, Nascimento HF, Santiago SB, Morelli ML, Barbosa MS (2017). Molecular technique for detection and identification of *Helicobacter pylori* in clinical specimens: a comparison with the classical diagnostic method. Journal Brasileiro de Patologia e Medicina Laboratorial 53(1):13-19. ID: biblio-893546.

Nguyen TH, Phan TB, Nguyen VB, Nguyen TVH, Phan TTB, Nguyen VB, Hoang TTH, Le TLA, Nguyen TTM, Vu SN (2017). Prevalence and risk factors of *Helicobacter pylori* infection in Muong children in Vietnam. Annals of Clinical Laboratory Research 5(1). DOI:10.21767/2386-5180.1000159.

Nurgalieva ZZ, Malaty HM, Graham DY, Almuchambetova R, Machmudova A, Kapsultanova D, Osato MS, Hollinger FB, Zhangabylov A (2002). *Helicobacter pylori* infection in Kazakhstan: effect of water source and household hygiene. The American Journal of Tropical Medicine and Hygiene 67(2):201-206.

Ozbey G, Hanafiah A (2017). Epidemiology, diagnosis and risk factors of Helicobacter pylori infection in children. Euroasian Journal of Hepato-Gastroenterology 7(1):34-39.

Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G (2014). Diagnosis of *Helicobacter pylori*: What should be the gold standard? World Journal of Gastroenterology 20(36):12847-12859.

Roland BC, Lee D, Salem A, Ibrahim S, Mori Y, Meltzer SJ (2016). Prevalence and associated risk factors of *Helicobacter pylori* negative gastritis. Journal of Gastrointestinal and Digestive System 6:392. doi:10.4172/2161-069X.1000392.

Sabh AA, El-Less FME (2017). Prevalence of common intestinal parasites in adult patients with chronic liver diseases associated with *Helicobacter pylori* Infection. Journal of the Egyptian Society of Parasitology 47(3):695-699.

Samson ES, Okeleke OJ, Richard AY, Gideon FT, Olutoyosi AL, Damilola O (2018). Screening for *Helicobacter pylori* infection among undergraduate students of a tertiary institution using serum antibody and stool antigen detection methods. Science and Technology Research 3(2):10. DOI: 10.26717/BJSTR.2018.03.000883.

Shi R, Xu S, Zhang H, Ding Y, Sun G, Huang X, Chen X, Li X, Yan Z, Zhang G (2008). Prevalence and risk factors for *Helicobacter pylori* infection in Chinese populations. *Helicobacter* 13:157-165.

Shiferaw G, Abera D (2019). Magnitude of *Helicobacter pylori* and associated risk factors among symptomatic patients attending at Jasmin internal medicine and pediatrics specialized private clinic in Addis Ababa city, Ethiopia. BMC Infectious Diseases 19:118. https://doi.org/10.1186/s12879-019-3753-5.

Simón CG, Andrés FCA, Tomás CG, Luis ACL, Héctor IGG, Santiago EM (2016). Prevalence of *Helicobacter pylori* and histopathological features in gastric biopsies from patients with dyspeptic symptoms at a referral center in Medellin. Revista Colombiana Gastroenterologia 31(1):9-14.

Subsomwong P, Miftahussurur M, Uchida T, Vilaichone RK, Ratanachuek T (2017). Prevalence, risk factors, and virulence genes of *Helicobacter pylori* among dyspeptic patients in two different gastric cancer risk regions of Thailand. PloS ONE 12(10):e0187113. DOI: 10.1371/journal.pone.0187113.

Suzuki S, Esaki M, Kusano C, Ikehara H, Gotoda T (2019). Development of *Helicobacter pylori* treatment: How do we manage antimicrobial resistance? World Journal of Gastroenterology 25(16):1907-1912.

Syam AF, Miftahussurur M, Makmun D, Nusi IA, Zulkhairi LH, Akil F, Uswan WB, Simanjuntak D, Uchida T, Adi P, Utari AP, Subsomwong YAA, Nasronudin P, Suzuki R, Yamaoka Y (2015). Risk factors and prevalence of *Helicobacter pylori* in five largest Islands of Indonesia: A Preliminary Study. PLoS ONE 23(2):14.

Tameshkel FS, Niya MHK, Kheyri Z, Azizi D, Roozafzai F, Khorrami S (2018). The evaluation of diagnostic and predictive values of *Helicobacter pylori* stool antigen test in Iranian patients with dyspepsia. Iranian Journal of Pathology 13(1):38-44.

Wong U, McLean LP (2016). Diagnosis and management of *Helicobacter pylori*. Journal of Clinical Gastroenterology and Treatment 2(1):1-6.

Zhu Y, Zhou X, Wu J, Su J, Zhang G (2014). Risk factors and prevalence of *Helicobacter pylori* infection in persistent high incidence area of gastric carcinoma in Yangzhong city. Gastroenterology Research and Practice Article ID 481365: 10 pages. DOI: 10.1155/2014/481365.

Vol. 15(6), pp. 334-340, June, 2021
DOI: 10.5897/AJMR2021.9475
Article Number: 43EDE6267148
ISSN: 1996-0808
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**African Journal of Microbiology Research**

*Full Length Research Paper*

# Resistance profile of *Salmonella* isolated from food sold in the streets of N'Djamena, Chad

**Mayoré Atéba Djibrine[1,2*], Bessimbaye Nadlaou[3], Bodering Alain[4], Evariste Bako[2], Abdelsalam Tidjani[3] and Nicolas Barro[2]**

[1]Higher National Institute of the Sahara and the Sahel in Iriba, Chad.
[2]Laboratory of Molecular Biology, Epidemiology and Surveillance of Bacteria and Food Transmitted Viruses (LaBESTA)/Doctoral School of Sciences and Technologies, Ouaga I Pr Joseph KI-ZERBO University, 03 P. O. Box 7021 Ouagadougou 03, Burkina Faso.
[3]Department of Public Health, Faculty of Human Health Sciences, University of N'Djamena, P. O. Box 1117, N'Djamena-Chad.
[4]Department of Chemistry-Biology-Geology, Faculty of Science and Technology, University of Doba, P. O. Box 03, Doba, Chad.

Received 2 January, 2021; Accepted 7 June, 2021

**The preparation and sale of street foods is booming in African cities in general and especially in N'Djamena. However, the hygienic failures observed during this activity constitute a source of contamination of these foods. The objective of this work is to determine the different serotypes of *Salmonella* isolated from food sold in the streets of N'Djamena in Chad and to test their sensitivity antibiotics. In total, 447 samples from 12 different types of food were collected and analyzed using standard food microbiology methods. The disk diffusion method was used to test the antibiotic susceptibility of *Salmonella* strains detected. The serotyping of the 5 strains of *Salmonella* allowed us to identify 3 serotypes namely *Salmonella* Mbandaka, *Salmonella* Idikan and *Salmonella* Anatum. The susceptibility profiles of the strains to antibiotics were varied. Resistance were observed with the antibiotics Amoxicillin + Clavulanic acid, Cefotaxime and Nalidixic acid. The most active antibiotics were Cefoxitne, Ciprofloxacin, Aztreonam, Imipenem and Choramphenicol with a rate of 100% sensitivity. However, the resistance of these strains to certain antibiotics is a real public health problem that calls out to food safety.**

**Key words:** Street food, contamination, *Salmonella*, serotype, resistance, Chad.

## INTRODUCTION

In Africa, street food vending and consumption have proliferated in the last three and a half decades (FAO, 2016). Street food can be defined as any ready to eat food or beverage sold and sometimes prepared in outdoor public spaces by vendors or cooked stationary outlet with or without indoor space to accommodate

*Corresponding author. E-mail: djibrine_ateba@yahoo.fr.

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consumers. This kind of foods concerns all genders, all age groups and all socio-professional categories (FAO, 2016). Street food is a regular source of income for millions of unskilled men and women in developing countries (FAO, 2010).

Studies in Africa and around the world have shown that dairy products, local drinks, rice with beans meat products and many others are contaminated with pathogens (Bawa, 2016; Bereda et al., 2016; Dossou et al., 2018). The pathogens involved in these foods are: *Escherichia coli*, *Salmonella* enterica, *Staphylococcus aureus*, coliforms, yeasts and molds at different levels (Bagré, 2016; Bawa, 2016; Bereda et al., 2016; Dossou et al., 2018; Soncy, 2018). High prevalence of *Salmonella* sp. were reported in studies of street foods in Botswana, Brazil, the Philippines and Morocco with 26% (Mrema et al., 2006), 25% (Dias et al., 2013), 15% (Manguiat and Fang, 2013) and 21.79% (Ed-dra et al., 2017). In Chad, Bessimbaye et al. (2013) in their study showed that most diarrheal diseases are due to coliforms and especially to pathovars of *E. coli*, *Salmonella* enterica and *Shigella*.

Antibiotics have since their discovery played a very important role in reducing the cases of disease and death related to microorganisms. These antibiotics have revolutionized the treatment of infectious diseases. However, the excessive use of these antimicrobials would be the reason for these multidrug resistance observed in certain strains (Mwambete and Peter, 2011; Makut et al., 2013; Rashed et al., 2013).

In a study conducted on foods in Morocco by Bouchrif et al. (2009), out of 104 isolates of *Salmonella* enterica including the serovars Infantis, Bredeney, Blokley, Typhimurium, Mbadaka, Branderup II and Kiambu, approximately 29% of the isolates were resistant to at least one antimicrobial. Resistance to tetracycline was most common (21%), followed by resistance to ampicillin (13%), amoxicillin + clavulanic acid (9%), streptomycin (7%), chloramphenicol (4%) and nalidixic acid (3.8%). Isolates of *S. Enteritidis* and *S. Typhimurium* obtained from chicken meat samples in Malaysia showed resistance to several antibiotics including erythromycin, penicillin and vancomycin (Thung et al., 2016). Antibiotic resistance in *Salmonella* has been associated with higher frequency and duration of hospital stays, longer disease, higher risk of invasive infection, and double increased risk of death within two years following infection (WHO, 2011).

The main aim of the present study is to determine the different serotypes of *Salmonella* isolated from food sold in the streets of N'Djaména in Chad and to test their sensitivity to antibiotics commonly used.

## MATERIALS AND METHODS

### Site and period of the study

The study took place in N'Djamena, the political capital of Chad between October 2014 and January 2018. Of the 10 districts in N'Djaména, eight were selected for this study.

### Sampling and sample processing

Approximately 447 samples were collected from the sites chosen for the conduct of the study (Figure 1). Random sampling was carried out and consisted of collecting samples of minced beef sandwich, the "ball" with okra sauce, rice with sorrel sauce, rice with tomato sauce, meat grilled mutton, fried fish, banana juice, avocado juice, raw mutton, raw beef, raw fish and grilled mutton seasoning. Quantities of 500 g (for solid products) were collected in sterile sachets and 500 ml (for liquid products) were collected in sterile bottles, placed in a cooler containing and sent to the laboratory, then analyzed within hours followed.

### Isolation and identification of *Salmonella*

For the detection of *Salmonella* a pre-enrichment in buffered peptone water for 24 h at 37°C was carried out, then two broths, Rappaport-Vassiliadis (41.5°C) and Muller-Kauffmann with Tetra Thionate-novobiocin (37°C) were used for enrichment. For isolation, Xylose Lysine Desoxycholate (XLD) and Hektoen agar media were used. The colonies presenting the typical appearance of *Salmonella* were subjected to biochemical tests namely Kligler-Hajna agar which consisted of looking for a slope (red), a pellet (yellow), with formation of gas and hydrogen sulphide (blackened agar). The second test was to look for the production or not of urease and indole. The presumed *Salmonella* colonies were confirmed by the API 20E gallery (BioMérieux, France).

### Serotyping of *Salmonella*

The serotyping of the *Salmonella* strains was carried out by the technique of direct agglutination on slide with the association of antigens (Ag) O (of the wall) and H (of the flagellum) according to the Kauffmann-White diagram (Kauffmann, 1966). The auto-agglutinating property or not of the *Salmonella* strains was detected using physiological water. Serotypes were determined by combining the "O" and "H" antigens obtained by agglutination test on a pure, non-self-agglutinating culture, isolated for 24 h on non-selective agar. For the strains on which auto-agglutination was not detected, we tested them successively with the O-polyvalent (OMA, OMB and OMC), O-monovalent and H (HMA, HMB, HMC and H1) according to the Kauffmann-White diagram (Kauffmann, 1966). The strains agglutinating with the OMA antiserum (OMA +) were tested with monovalent O sera [O: 1.2], [O: 4.5], [O: 3, 10.15], [O: 9]. The OMB + strains were tested with a monovalent O agglutinating serum O: 6, 7, 8. All strains agglutinating with antisera from groups C and D were tested with Vi antiserum. From the Svend Gard (SG) soft agar, we used H: a flagellar antisera, for the strains agglutinated with the antisera of groups A, the antisera H: b, H: i and H: g, m for those of group B and H: d for those of group D / Vi positive, then H: g, m and H: p for the other strains of group D / Vi-negative. The antigenic formula and the reading of the serotyping results were carried out using the Kauffman-White minor table (Grimont and Weill, 2007; Guibourdenche et al., 2010).

### Performing the antibiogram

#### *Preparation of the inoculum*

For performing the antibiogram, the agar diffusion method was used (Bauer et al., 1966). Thus, the strains of *Salmonella* sp

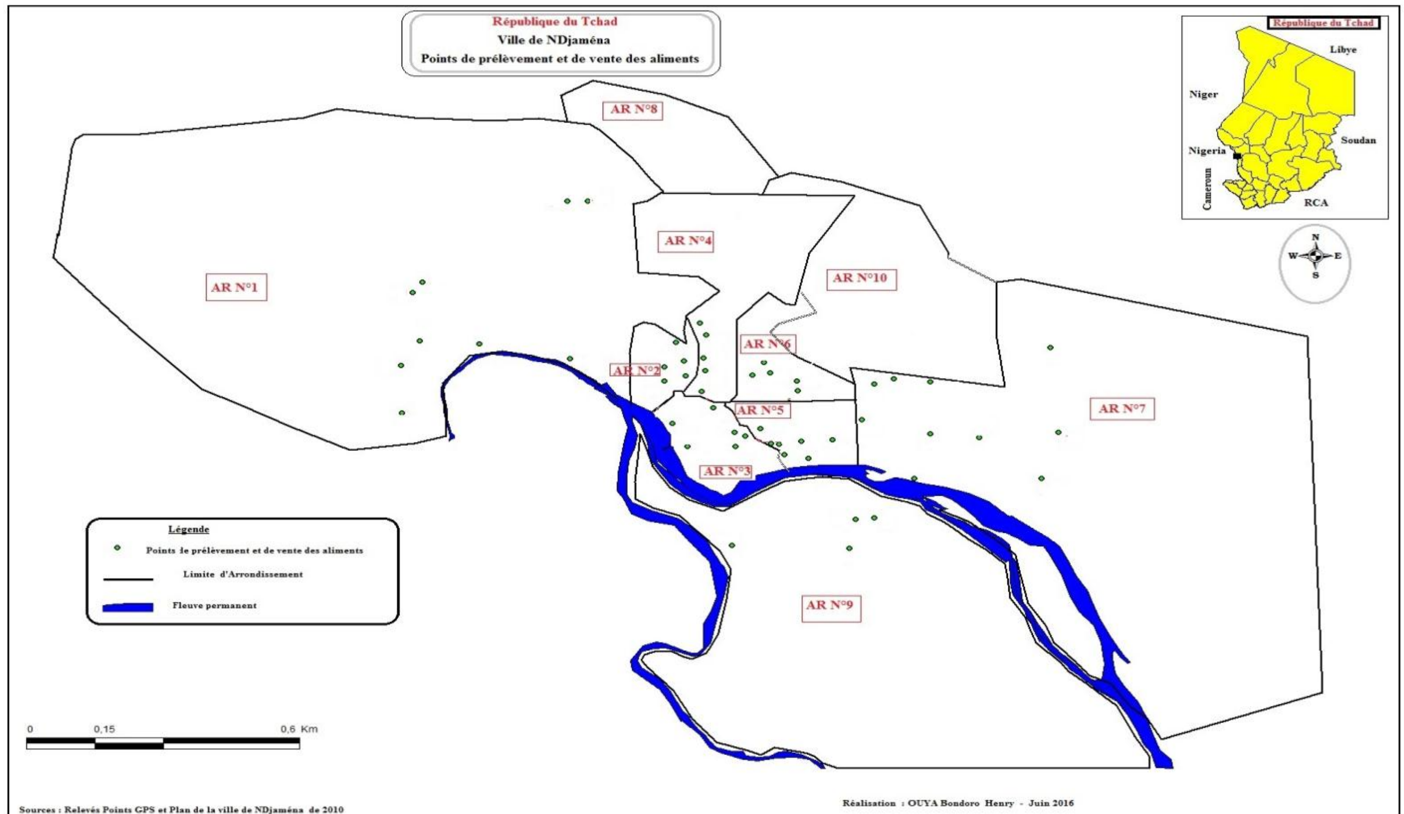


**Figure 1.** Sampling sites.

isolated previously from food samples were subcultured on Mueller Hinton agar (Liofilchem, Italy) then incubated at 37°C. After incubation for 18-24 h, a few bacterial colonies were suspended in physiological water. The resulting suspension was calibrated to the MacFarland 0.5 standard (~ 108 CFU / ml) using a densimat.

#### *Inoculation of Salmonella* sp.

Agar medium (Mueller Hinton) was as described by Kirby-Bauer. Flood seeding was carried out using 3 to 5 ml of inoculum. The excess bacterial suspension was removed with a micropipette. The Petri dish was then kept for 15 min at room temperature for drying under a microbiological safety post (PSM). Antibiotic discs were then placed at an equal distance on the inoculated agar medium using sterile forceps. A light pressure was exerted on the discs in order to consolidate their adhesion to the agar medium. The Petri dishes containing the antibiotic discs were finally kept at room temperature before being incubated for 24 h at 37°C.

#### *Choice of antibiotics to test*

The antibiotics tested were those commonly used for treatment in human and veterinary medicine in Chad. Thus, the following range of antibiotics have been tested on the strains of *Salmonella*: two Cephalosporins (Cefotaxime (5 µg), Cefoxitin (30 µg)); one Fluoroquinolone (Ciprofloxacin (5 µg)); one Quinolones (Nalidixic acid (30 µg)); one Penicillin (Amoxicillin + Clavulanic Acid (20 / 10 µg)); one Monobactam (Aztreonam (30 µg)); one Carbapeneme (Imipeneme (10 µg)); one Phenicol (Chloramphenicol (30 µg)).

#### *Reading*

After incubation, sterile circular culture areas were observed around some of the antibiotic discs. The diameter of these zones or diameter of inhibition is proportional to the antibacterial activity of the antibiotic. Thus, these inhibition diameters were measured using a caliper. The results were interpreted according to the recommendations of AC-FSM/EUCAST (2016).

## RESULTS

The different sampling sites are made up of markets, primary schools, avenues, the administrative area, high schools, stations and the industrial area (Figure 1). The different foods analyzed are based on cereals, meat and fish (Table 1). The different strains isolated showed varying levels of sensitivity and resistance to the antibiotics tested (Table 3).

### Presentation of meals analyzed during the study

#### *Prevalence and serotypes of Salmonella in street foods in N'Djaména*

Out of the 447 samples from 12 different types of food analyzed, we noted the presence of *Salmonella* in 5

**Table 1.** Description of the types of food analyzed.

| N° | Type of food | Description of the food |
|---|---|---|
| **Cereal-based foods** | | |
| 01 | Minced beef sandwich | Bread loaded with minced meat cooked in a sauce with tomato, potato, onions, mayonnaise, aroma of oil and salt |
| 02 | "Boule" with okra sauce | Rice flour dough prepared and eaten with okra meat sauce (beef or mutton) |
| 03 | Rice with sorrel sauce | White rice boiled and served with sorrel sauce with meat (beef or mutton) |
| 04 | Rice with tomato sauce | White rice boiled and served with a tomato sauce made with meat (beef or mutton) or fish |
| **Meat-based foods** | | |
| 05 | Grilled mutton | Meat grilled on a wire mesh placed on a masonry stove containing a wood fire. The grilled meat is eaten with a seasoning consisting of salt, peanut powder, chilli pepper and flavoring |
| 06 | Raw mutton | Raw mutton |
| 07 | Raw beef | Raw beef |
| **Fish based foods** | | |
| 08 | Fried fish | Fried fish |
| 09 | Raw fish | Raw fish |
| **Fruit based foods** | | |
| 10 | Banana juice | The flesh of the fruit is mixed using a Moulinex. The main ingredients are ice, sugar and powdered milk |
| 11 | Avocado juice | The flesh of the fruit is mixed using a Moulinex. The main ingredients are ice, sugar and powdered milk |
| **Other** | | |
| 12 | Grilled mutton seasoning | This seasoning is composed of salt, peanut powder, chilli pepper and flavoring |

samples (Table 2). It should be remembered that the 5 contaminated samples were those of rice with sorrel sauce. Serotyping made it possible to identify 3 *Salmonella* serotypes as shown in Table 2.

### Antibiotic sensitivity of *Salmonella sp.*

Table 3 the antibiotic sensitivity profile of the *Salmonella* strains isolated in this study. The different strains isolated showed varying levels of sensitivity and resistance to the antibiotics tested. On the one hand, the isolates were all susceptible to Ciprofloxacin, Cefoxitin, Chloramphenicol, Aztreonam and Imipenem. On the other hand, the phenotypic resistance profile of the *Salmonella* reveals that the only strain of S. Idikan showed resistance to both Amoxicillin + Clavulanic Acid and Nalidixic Acid. Finally, one in two strains of S. Anatum exhibited resistance to Cefotaximé.

## DISCUSSION

Analysis of the different foods showed that the majority were not contaminated with *Salmonella*, this result is consistent with those of many studies conducted on street foods (El Marnissi et al., 2012; Diane et al., 2017; Boko et al., 2017; Dossou et al., 2018; Mayoré et al., 2018; Doutoum et al., 2019). In rice with sorrel sauce, there is a strong presence of *Salmonella* as in several other studies (Bereda et al., 2016; Justin et al., 2018). The presence of *Salmonella* in the rice sorrel sauce could come from the sorrel leaves which are used for the preparation of the sauce if the cooking is not done well or from cross contamination following poorly washed utensils

**Table 2.** Prevalence of *Salmonella* in street foods in N'Djaména.

| Type of food | Number | *Salmonella* serotypes | | |
|---|---|---|---|---|
| | | ***S.* Mbandaka** | ***S.* Idikan** | ***S.* Anatum** |
| Minced Beef Sandwich | 42 | 00 | 00 | 00 |
| "Ball" with okra sauce | 42 | 00 | 00 | 00 |
| Rice with sorrel sauce | 39 | 02 | 01 | 02 |
| Rice with tomato sauce | 36 | 00 | 00 | 00 |
| Grilled mutton | 49 | 00 | 00 | 00 |
| Fried fish | 31 | 00 | 00 | 00 |
| Banana juice | 31 | 00 | 00 | 00 |
| Avocado juice | 31 | 00 | 00 | 00 |
| Raw mutton | 41 | 00 | 00 | 00 |
| Raw beef | 44 | 00 | 00 | 00 |
| Raw fish | 33 | 00 | 00 | 00 |
| Grilled mutton seasoning | 28 | 00 | 00 | 00 |
| **Total** | **447** | **02** | **01** | **02** |

**Table 3.** Susceptibility profile of strains of *Salmonella* sp.

| Antibiotiques | Sérotype de *Salmonella* | | | | | |
|---|---|---|---|---|---|---|
| | ***S.* Mbandaka (n = 2)** | | ***S.* Idikan (n = 1)** | | ***S.* Anatum (n = 2)** | |
| | **S** | **R** | **S** | **R** | **S** | **R** |
| **Cephalosporins** | | | | | | |
| Cefotaxime | 02 | 00 | 01 | 00 | 01 | 01 |
| Cefoxitin | 02 | 00 | 01 | 00 | 02 | 00 |
| **Quinolone** | | | | | | |
| Nalidixic Acid | 02 | 00 | 00 | 01 | 02 | 00 |
| **Fluoroquinolones** | | | | | | |
| Ciprofloxacin | 02 | 00 | 01 | 00 | 02 | 00 |
| **Penicillin** | | | | | | |
| Amoxicillin + Clavulanic Acid | 02 | 00 | 00 | 01 | 02 | 00 |
| **Monobactam** | | | | | | |
| Aztreonam | 02 | 00 | 01 | 00 | 02 | 00 |
| **Carbapenem** | | | | | | |
| Imipenem | 02 | 00 | 01 | 00 | 02 | 00 |
| **Phenicol** | | | | | | |
| Chloramphenicol | 02 | 00 | 01 | 00 | 02 | 00 |

S = sensitive ; R = resistant. ; n = *Salmonella* strains number.

used for the service (Mayoré, 2019). In food, the presence of *Salmonella* is worrying for the health of the consumer. According to the World Health Organization, *Salmonella* (non-tyhoid) is responsible for tens of millions of cases of water-borne and food-borne illnesses in humans each year around the world (WHO, 2013).

The serotype Anatum identified in rice with sorrel sauce were also found in Carcass sponge, Bone meal, Raw chicken and Process chicken (Shafini et al., 2017; Da Cunha-Neto et al., 2017). *S.* Anatum is frequently detected in cattle and in faeces, skin, lymph nodes, meat fluids, and carcasses of dairy cattle and beef in the southern United States (Kunze et al., 2008), such as in the case of beef from Mexico (Varela-Guerrero et al., 2013), Namibia (Shilangale et al., 2015) and South Africa (Madoroba et al., 2016). In France for example, between

2007 and 2012, the data collected on the food samples analyzed underline an almost constant evolution in the number of strains of the *S.* Mbandaka serovar (Renaud et al., 2015).

Foodborne illness caused by non-typhoid salmonellosis is a major public health problem around the world. However, examination of the resistance profile to different classes of antibiotics performed on isolates of the different *Salmonella* serotypes isolated from food samples shows some resistance to one class of antibiotics. Since these pathogens could be transmitted through the environment or contaminated water, their presence could have an impact on public health. Our results show resistance of *Salmonella* to Amoxicillin, Cefotaxime and Nalidixic Acid. An earlier study carried out in Chad revealed resistance to at least one of these antibiotics (Djim-Adjim et al., 2013) and also studies carried out in several other countries have made similar findings (Thong and Modarressi, 2011; Bagré et al. 2014; Bawa et al. 2015). Quinolones are generally the drugs of choice for the treatment of invasive *Salmonella* infections in adults, and resistance to nalidixic acid may invalidate treatment with quinolone. The total sensitivity of our isolates to Ciprofloxacin, Cefoxitin, Chloramphenicol, Aztreonam and Imipenem has also been variously reported by Bagré et al. (2014); Bawa et al. (2015) and Bsadjo (2015). The resistance of *Salmonella* strains to different antibiotics could be explained by the fact that these antibiotics are often used inappropriately in unregulated doses, thus becoming a factor favoring the adaptation of strains to antibiotics (Iroha et al., 2011; Carvalho et al., 2013; Li et al., 2013).

## Conclusion

The study showed that the strains of *Salmonella* sp., Isolated from street foods, are of different serotypes and resistant to the antibiotics commonly used in human medicine in Chad. This particularly pronounced resistance to antibiotics of the quinolone family is a real concern for public health. The prescription and use of antibiotics, whether for the treatment of animals or humans, must be the subject of mature studies in order to better control the proliferation of antibiotics.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors warmly thank the Research Project and Support for Street Food Safety ("PRASAR"), who financially supports us throughout the laboratory phase. Special thanks also go to the site of the Research Institute of Livestock Development ("IRED") who offered us a technical platform to a higher level for our research.

## REFERENCES

Antibiogram Committee of the French Society of Microbiology, European Committee on Antimicrobial Susceptibility Testing (AC-FSM/EUCAST) (2016). Recommandations 2016. Version 1.0 P.117.

Bagré TS (2016). Assessment of the contamination of dairy products consumed in Burkina Faso by residues of antibiotics, pathovars of Escherichia coli and Salmonella enterica. Unique Thesis - Ouaga I Pr Joseph KI-ZERBO University P.156.

Bagré TS, Kagambèga A, Bawa IH, Bsadjo TG, Dembélé R, Zongo C, Savadogo A, Aggad H, Traoré AS, Barro N (2014). Antibiotic susceptibility of *Escherichia coli* and *Salmonella* strains isolated from raw and curds milk consumed in Ouagadougou and Ziniaré, Burkina Faso. African Journal of Microbiology 8:1012-1016.

Bauer AW, Kirby WMM, Sherries JC, Turck M (1966). Antibiotic susceptibility testing. American Journal of Pathology 45:493-496.

Bawa IH (2016). Phenotypic, molecular and antibiotic resistance characterization of strains of Salmonella spp. and Escherichia coli isolated from samples of meat sold in markets and samples from patients. Unique Thesis - Ouaga I Pr Joseph KI-ZERBO University P.165.

Bawa IH, Bsadjo TG, Bagré TS, Bouda SC, Konaté A, Bako E, Kagambèga A, Zongo C, Somda M, Savadogo A, Traoré AS, Barro N (2015). Antimicrobial susceptibility of *Salmonella* enterica strains isolated from raw beef, mutton and intestines sold in Ouagadougou, Burkina Faso. Journal of Applied Biosciences 95:8966-8972.

Bereda TW, Emerie YM, Reta MA, Asfaw HS (2016). Microbiological safety of street vended Foods in Jigjiga city, Eastern Ethiopia. Ethiopian Journal of Health Sciences 26(2):161-170.

Bessimbaye N, Tidjani A, Moussa AM, Brahim BO, Mbanga D, Ndoutamia G, Sangaré L, Barro N, Traoré AS (2013). Gastroenteritis with *Escherichia coli* in pediatric hospital in N'Djamena-Tchad. Journal of Applied Biology and Biotechnology 1:13-17.

Boko KC, Gangnito M, Soumanou ST, Sessou P, Ulbad PT, Aguidissou ON, Kpodekon MT, Souaïbou F (2017). Microbial Quality of "Tchachanga", a Barbecued Mutton Sold in Benin. Advances in Microbiology 7(8):633-640.

Bouchrif B, Paglietti B, Murgia M, Piana A, Cohen N, Ennaji MM, Rubino S, Timinouni M (2009). Prevalence and antibiotic-resistance of *Salmonella* isolated from food in Morocco. Journal of Infection in Developing Countries 3(1):35-40.

Bsadjo TG (2015). Characterization of strains of Escherichia coli and Salmonella sp. contaminating refreshing drinks and ice cream in the city of Ouagadougo, Burkina Faso. Unique Thesis - Ouaga I Pr Joseph KI-ZERBO University P 189.

Carvalho FCT, Sousa OV, Carvalho EMR, Hofer E, Vieira RHSF (2013). Antibiotic Resistance of *Salmonella* spp. Isolated from Shrimp Farming Freshwater Environment in Northeast Region of Brazil. Journal of Pathogens P.5.

Da Cunha-Neto A, Castro VS, Carvalho LA, Dália DPR, Mano SB, De Souza FEE, Conte-Junior CA (2017). Serotypes and antimicrobial resistance profiles of *Salmonella* isolated from fresh beef processing and chilled fresh beef samples produced and marketed in the metropolitan region of Cuiabá, in the State of Mato Grosso, Brazil. African Journal of Microbiology Research 11(45):1626-1631.

Diane DE, Akmel DC, Tano K, Assidjo NE, Akaki D, Montet D (2017). Quantitative Assessment of the Risk Linked to the Consumption of Braised Beef Meat "*Choukouya*" Contaminated with Pathogenic *Clostridium perfringens* in Côte D'Ivoire. Food and Nutrition Sciences 8(12):1137-1155.

Dias FS, Ramos CL, Amanda RAA, Ramos CL, Santos RRMM, Schwan RF (2013). Identifcation of *Salmonella* isolated from pork sausage and evaluation of thermal and antimicrobial resistance of isolates. African Journal of Microbiology Research 7(44):5070-5075.

Djim-Adjim T, Diguimbaye D, Sophie AG, Frederique M, Brisabois A, Elgroud R, Millemann Y (2013). Prevalence and antimicrobial resistance of non-typhoidal *Salmonella* serotypes isolated from laying

hens and broiler chicken farms in N'Djamena, Chad. Veterinary Microbiology 166(1-2):293-298.

Dossou BR, Nassaya S, Karou DS, Soncy K, Kagni-Dossou M, Anani K, Ameyapoh Y (2018). Assessment of the Safety of Cooked Meals Sold on the Outskirts of the Streets of the City of Kara in Togo. Journal of Scientific Research of the University of Lomé (Togo) 20(3):71-80.

Doutoum AA, Tidjani A, Markhous NA, Kimassoum D, Nadlaou B (2019). Microbiological Quality Assessment of the Main Food Consumed in Collective Catering in the City of N'Djamena-Chad. Journal of Tropical Medicine and Health P 139.

Ed-dra A, Rhazi FF, El Allaoui A, Aboulkacem A (2017). Factors influencing the bacteriological quality of sausages sold in Meknes city, Morocco. International Food Research Journal 24(3):933-938.

El Marnissi B, Bennani L, El oulali lalami A, Aabouch M, Belkhou R (2012). Contribution to the study of the microbiological quality of foodstuffs marketed in Fès-Boulemane. Journal of Industrial, Sanitary and Environmental Microbiology 6(1):98-117.

Food and Agriculture Organization of the United Nations (FAO) (2010). The State of Food Insecurity in the World. FAO.

Food and Agriculture Organization of the United Nations (FAO) (2016). Street food in urban Ghana : A desktop review and analysis of findings and recommendations from existing literature. Report. P.90.

Grimont PAD, Weill FX (2007). Antigenic formulae of the *Salmonella* serovars. In WHO (Eds.).World Health Organization Collaborating Center for Reference and Research on *Salmonella*, 9th edition. Pasteur Institute, Paris P 166.

Guibourdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemuhl J, Grimont PA, Weill FX (2010). Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. Research in Microbiology 161(1):26-29.

Iroha IR, Ugbo EC, Ilang DC, Oji AE, Ayogu TE (2011). Bacteria contamination of raw meat sold in Abakaliki, Ebonyi State Nigeria. Journal of Public Health and Epidemiology 3(2):49-53.

Justin OBJ, Lilly NP, Theodore MBM, Antoine L, Tresor M, Fideline N, Espoir I, Betu M (2018). Bacteriological quality of street foods vended in Bukavu City: Potential health risks to consumers of south Kivu province, Eastern D.R. Congo. Bacterial Empire 1(1):13-21.

Kauffmann F (1966). On the history of *Salmonella* research. Zentralblatt für Bakteriologie 201(1):44-48.

Kunze DJ, Loneragan GH, Platt TM, Miller MF, Besser TE, Koohmaraie M, Stephens T, Brashears MM (2008). *Salmonella enterica* burden in harvest-ready cattle populations from the southern high plains of the United States. Applied and Environmental Microbiology 74(2):345-351.

Li R, Lai J, Wang Y, Liu S, Li Y, Liu K, Shen J, Wu C (2013). Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. International Journal of Food Microbiology 163(1):14-18.

Madoroba E, Kapeta D, Gelaw AK (2016). *Salmonella* contamination, serovars and antimicrobial resistance profiles of cattle slaughtered in South Africa. Onderstepoort Journal of Veterinary Research 83(1):1-8.

Makut MD, Nyam MA, Obiekezie SO, Abubakar AE (2013). Antibiogram of bacteria isolated from kunun-zaki drink sold in Keffi metropolis. American Journal of Infectious Diseases 9(3):71-76.

Manguiat LS, Fang TJ (2013). Microbiological quality of chicken-and pork-based street vended foods from Taichung, Taiwan and Laguna, Philippines. Food Microbiology 36(1):57-62.

Mayoré AD (2019). Health characteristics and antibiotic resistance of pathogenic strains isolated from street foods in the city of N'Djaména (CHAD). Unique Thesis - Ouaga I Pr Joseph KI-ZERBO University P 191.

Mayoré AD, Tidjani A, Ngandolo BN, Nadlaou B, Barro N (2018). Microbiological quality of some street foods in N'Djamena, Chad: case of sandwiches. International Journal of Biological and Chemical Sciences 12(3):1113-1122.

Mrema N, Mpuchane S, Gashe BA (2006). Prevalence of *Salmonella* in raw minced meat, raw fresh sausages and raw burger patties from retail outlets in Gaborone, Botswana. Food Control 17(3):207-212.

Mwambete KD, Peter A (2011). Microbiological Quality of Juice Beverages Available in Dar Es Salaam and Resistance Profiles of Microbial Contaminants. East and Central African Journal of Pharmaceutical Sciences 14(3):81-88.

Rashed N, Aftab U, Azizul H, Saurab KM, Mrityunjoy A, Majibur R (2013). Microbiological study of vendor and packed fruit juices locally available in Dhaka city, Bangladesh. International Food Research Journal 20(2):1011-1015.

Renaud L, Moury F, Leclerc V, Bohnert M, Cadel-Six S, Brisabois A (2015). Monitoring of Salmonella in the food chain for the detection of emergencies in France. Epidemiological Bulletin, Animal Health and Food 68:11-16.

Shafini AB, Son R, Mahyudin NA, Rukayadi Y, Tuan ZTC (2017). Prevalence of *Salmonella* spp. in chicken and beef from retail outlets in Malaysia. International Food Research Journal 24(1):437-449.

Shilangale R, Kaaya G, Chimwamurombe P (2015). Prevalence and characterization of *Salmonella* isolated from beef in Namibia. European Journal of Nutrition and Food Safety 5:267-274.

Soncy K (2018). Study of the microbial contamination of foods for the control of risk of poisoning: case of out-of-home catering in the municipality of Lomé (Togo). Unique Thesis -Lomé University 144 p.

Thong KL, Modarressi S (2011). Antimicrobial resistant genes associated with *Salmonella* from retail meats and street foods. Food Research International 44(9):2641-2646.

Thung TY, Mahyudin NA, Basri DF, Wan CWJMR, Nakaguchi Y, Nishibuchi M, Radu S (2016). Prevalence and antibiotic resistance of *Salmonella* Enteritidis and *Salmonella* Typhimurium in raw chicken meat at retail markets in Malaysia. Poultry Science 95(8):1888-1893.

Varela-Guerrero JA, Talavera-Rojas M, Gutiérrez-Castillo AC, Reyes-Rodríguez NE, Vázquez-Guadarrama J (2013). Phenotypic–genotypic resistance in *Salmonella* spp. Isolated from cattle carcasses from the north-central zone of the State of Mexico. Tropical Animal Health and Production 45(4):995-1000.

World Health Organization (WHO) (2013). *Salmonella* (non-typhoidal). http://www.who.int/mediacentre/ factsheets/fs139/en/.

World Health Organization/Regional Office for Europe (WHO/ROE) (2011). Tackling antibiotic resistance from a food safety perspective in Europe. Report P 88.

Vol. 15(6), pp. 341-348, June, 2021
DOI: 10.5897/AJMR2021.9508
Article Number: C866F0067154
ISSN: 1996-0808
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African Journal of Microbiology Research

*Full Length Research Paper*

# Prevalence and factors associated with *Staphylococcus aureus* nasal colonisation in orthopaedic patients at a tertiary care hospital in Kenya

**David Githiomi Mwaura[1], Sitati Fred Chuma[2]* and Edward Gakuya[2]**

[1]Kitui County Hospital, Kitui, Kenya.
[2]Department of Orthopaedic Surgery, Faculty of Medicine, University of Nairobi, Kenya.

Received 22 February 2021; Accepted 20 April, 2021

***Staphylococcus aureus* is an important organism in orthopaedic practice as it is the most common cause of orthopaedic infections including surgical site infections (SSIs), osteomyelitis and septic arthritis. Carriers of *S. aureus* are predisposed to developing invasive staphylococcal infections. Knowledge of a patient's carrier status before surgery together with interventions to eliminate the carrier state have been shown to reduce post-operative infections by *S. aureus*. A cross-sectional study carried out at Kenyatta National Hospital orthopaedic wards from 1 June 2019 to 30 September 2019. To determine the prevalence and factors associated with nasal colonisation by *S. aureus* among patients who have been admitted to undergo surgery. Consecutive sampling was done until the required sample size was achieved. Nasal swabs were taken from patients at admission for culture. Data concerning comorbid conditions as well as healthcare associated risk factors was collected. The overall prevalence of colonisation by *S. aureus* at admission was found to be 24.7% whereas the overall prevalence of colonisation by Methicillin Resistant *S. aureus* (MRSA) was found to be 3.03%. The prevalence of colonisation by *S. aureus* is high amongst patients being admitted to orthopaedic wards at Kenyatta National Hospital when compared with previous studies and amongst these are those who are colonised by MRSA. The prevalence of MRSA calls for the need of screening programmes to curtail spread within hospital and community settings.**

**Key words:** *Staphylococcus aureus*, prevalence, nasal colonization, associated factors.

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first documented in 1960 and was mainly found in hospital settings with a few community outbreaks. Currently, the prevalence of MRSA is increasing exponentially and has become a global concern. Knowledge of a patient's *S. aureus* carrier status and subsequent decolonisation has been shown to decrease the occurrence of infective complications after orthopaedic surgery with between 56 and 75% reduction of *S. aureus* surgical site infections (SSIs), 29 and 100% reduction of SSIs due to MRSA and 29 and 81% reduction in all SSIs (Chen et al., 2013; Jeans et al.,

*Corresponding author. E-mail: fredsitati@yahoo.com.

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2018; Ning et al., 2020).

In patients who develop *S. aureus* infections bacteria cultured from the site match (by phage typing) those from the nares in 85% of the cases suggesting an endogenous source of infection (Reighard et al., 2009). Kalmeijer et al. (2000) identified nasal carriage of *S. aureus* as the only independent risk factor for *S. aureus* SSI after orthopaedic implant surgery. Staphylococcal infections after orthopaedic operations are associated with greater mortality rates and increased healthcare expenditure as a result of the need for revision procedures and greater length of stay in hospital (Schmidt et al., 2015).

Results from this study will provide baseline data on the magnitude of *S. aureus* colonisation in orthopaedic patients as well as identify factors predictive of nasal colonisation.

This study aimed to determine the prevalence of nasal colonisation by *S. aureus* among orthopaedic patients being admitted for surgery as well as the risk factors associated with nasal colonisation by Methicillin Resistant *S. aureus* (MSSA) and Methicillin Sensitive *S. aureus* (MRSA).

## METHODOLOGY

This cross-sectional study was conducted at Kenyatta National Hospital (KNH) Orthopaedic Wards. KNH is a metropolitan, tertiary, teaching and referral hospital situated at about 5 km from Nairobi city centre. It has a 2000 bed capacity with the orthopaedic wards having a capacity of approximately 300 patients. KNH is a major referral hospital serving East and Central Africa.

Patients aged 18 years and above admitted to the orthopaedic wards between 1st June 2019 and 30th September 2019 and had been assigned for surgery in which orthopaedic implants were to be used.

The study excluded:

(1) Patients who were currently receiving antibiotics or had been on antibiotics during the preceding two weeks.
(2) Patients in whom nasal manipulation is contraindicated.
(3) Patients with active MRSA or MSSA infections.
(4) Patients with upper respiratory tract infections.

All eligible patients were enrolled until the required sample size was obtained.

### Sample size

Kenyatta National Hospital has 3 orthopaedic wards that each has a theatre list with approximately 3 patients per day. Going by previous ward records the study envisages a population of 500 patients within the study period (assuming a recruitment success rate of 2 patients per ward per day). The sample size was calculated using Krejcie and Morgan (1970) formula as follows:

$$s = Z^2(1 - \infty/2) \times NP(1 - P) / d^2 (N - 1) + Z^2(1 - \infty/2) P(1 - P)$$

where s = sample size to be determined, $Z^2 (1-\infty/2)$ is the standard error of the mean corresponding to a 95% confidence interval and the corresponding value from a t-table is 1.96, N = Estimated population size, P is the expected prevalence of the event to occur. Value of P was 0.3. d is the target margin of error which will be 5% (0.05) to increase precision.

Therefore, the sample size becomes:

$$s = 1.96^2 \times 500 \times 0.3 (1 - 0.3) / 0.05^2 \times 499 + 1.96^2 \times 0.3 \times 0.7$$

Hence s = 198.

### Data collection and analysis

Patients were recruited into the study; data was collected using a questionnaire administered by the interviewer and nasal swab samples taken within 24 h of their admission to the orthopaedic wards.

All enrolled patients were interviewed using a questionnaire, which assessed patient biodata and socio demographic information, co morbidities and risk factors for colonisation by *S. aureus*. Participant’s weight and height were measured to calculate body mass index (BMI). They were then examined for wounds (such as abrasions, lacerations, draining sinuses, etc.) and signs of soft tissue infection as evidenced by redness or a purulent discharge. This study utilized single site swabbing due to financial limitations. However, this study is useful as it determined the prevalence of nasal colonisation with *S. aureus* within 24 h of admission to reduce chances of nosocomial transmission. All samples were taken by qualified personnel.

## RESULTS

Nasal swab collection was adapted from CDC guidelines for collection of specimens using sterile cotton swabs moistened with sterile water for patient comfort (US Centers for Disease Control and Prevention, 2011). The entire procedure was done by a trained laboratory technician.

The swabs were then inoculated onto sheep blood agar (Oxoid Ltd. Hampshire, UK) within 1 h of sample collection. Plates were incubated at 37°C and examined for growth after 24 to 48 h. Isolates were confirmed as *S. aureus* based on colonial morphology, Gram staining, coagulase and catalase tests. Microscopy was done at University of Nairobi Paediatrics Laboratory.

Data was entered into an excel sheet and analysed using Statistical Package for the Social Sciences (SPSS) version 25. The risk factors and sociodemographic characteristics were analysed at univariate and multivariate levels with the use of Chi square tests. Odds ratio as well as 95% confidence intervals were calculated. A p-value < 0.05 was considered significant. Analysis of results was done with the help of a statistician. The Paediatrics Laboratory, University of Nairobi was used.

## Ethical considerations

WHO International ethical guidelines for biomedical research involving human subjects were followed throughout the study. Ethical approval was sought prior to commencing the study from the KNH/UON Ethics, Research and Standards Committee and the Department of Orthopaedic Surgery, University of Nairobi. Written

**Table 1.** Characteristics of the study participants.

| Characteristic | Males | Females | Total |
|---|---|---|---|
| **Population size, *S. aureus* carriage and age** | | | |
| Number of Participants | 167 (84.3) | 31 (15.7) | 198 (100) |
| Age, years, mean (range) | 34 (18-88) | 39(19-68) | 35 (18-88) |
| **Education** | | | |
| Primary | 61 (36.5) | 10 (32.3) | 71 (35.9) |
| Secondary | 82 (49.1) | 14 (45.2) | 96 (48.4) |
| Tertiary | 24 (14.4) | 7 (22.6) | 31 (15.7) |
| **Body Mass Index (BMI)** | | | |
| Underweight | 37 (22) | 2 (6) | 39 (20) |
| Normal | 106 (63) | 12 (39) | 118 (60) |
| Overweight | 19 (11) | 9 (29) | 28 (14) |
| Obese | 5 (3) | 8 (26) | 13 (7) |
| **Risk factors for *S. aureus* (MRSA and MSSA) colonisation** | | | |
| Smoking | 42 (25.1) | 1 (3.2) | 43 (21.7) |
| Diabetes | 4 (2.4) | 4 (12.9) | 8 (4) |
| HIV | 6 (3.6) | 6 (19.4) | 12 (6.1) |
| Admitted to a healthcare facility in the past one year | 16 (9.6) | 4 (12.9) | 20 (10.1) |
| Used any antibiotics in the past 3 months | 59 (35.3) | 16 (51.6) | 75 (37.9) |
| Skin or soft tissue infections in the past one year | 12 (7.2) | 5 (16.1) | 17 (8.6) |
| Previously admitted to ICU | 1 (0.6) | 0 | 1 (0.5) |
| Open wounds | 64 (38.3) | 11 (35.5) | 75 (37.9) |
| Outpatient clinic in the past one year | 29 (17.4) | 15 (48.4) | 44 (22.2) |
| Household members ≤ 4 | 121 (72.5) | 20 (64.5) | 141 (71.2) |
| Household members > 5 | 46 (27.5) | 11 (35.50 | 57 (28.8) |
| Soft tissue infection | 14 (8.4) | 5 (16.1) | 19 (9.6) |

[1]Unless otherwise indicated, data are no. (%) of participants by gender.

informed consent prior to participant enrolment was obtained. Strict confidentiality was observed throughout the period of the study.

## Patient characteristics

A total of 198 patients were recruited into the study from 1st June 2019 to 30th September 2019. Of these 167 were male and 31 were female. The male to female ratio was 5.4:1. The mean age was 35, ranging from 18 to 88 years.

Forty-three (21.7%) patients were smokers, eight (4%) were diabetic and 12 (6.1%) were (Human Immunodeficiency virus) HIV positive. Of the 198 patients, 20 (10.1%) had been admitted to a healthcare facility in the past year, 44 (22.2%) had attended a form of outpatient clinic in the past year and 75 (37.9%) had used antibiotics in the preceding 3 months prior to admission. Majority of patients (48.4%) had attained at least secondary school level education and 118 (60%) were of normal BMI (Table 1).

## Nasal carriage of *S. aureus*

A total of 49/198 patients had *S. aureus* isolated from the nasal swab samples collected on admission to the hospital. Of the 49, 43 (87.8%) were MSSA and 6 (11.9%) were MRSA. The overall prevalence of MRSA among the study participants was 6/198 (3.03%) whereas the overall prevalence of S. *aureus* nasal colonisation was 49/198 (24.7%). The mean age for the patients colonised by *S. aureus* was 34.8 (SD=9.7) years, those colonised with MRSA was 32.0 (SD=9.1) years, while those colonised with MSSA was 35.2 (SD=9.8). Majority of those colonised by MSSA had heavy growth of the bacteria whereas those colonised by MRSA predominantly had light growth of bacteria.

Of the patients colonised by MRSA, one was female and 5 were male. Only one had no risk factors, 4 had previously visited an outpatient clinic and had antibiotics

**Table 2.** Characteristics of study participants with MRSA and MSSA.

| Characteristics | N | Colonised n (%) | |
|---|---|---|---|
| | | MRSA | MSSA |
| **Age** | | | |
| 18-30 | 19 | 2 (33.3) | 17 (39.5) |
| 31-60 | 29 | 4 (66.7) | 25 (58.1) |
| >60 | 1 | 0 (0.0) | 1 (2.3) |
| **Sex** | | | |
| Male | 42 | 5 (83.3) | 37 (86) |
| Female | 7 | 1 (16.7) | 6 (14) |
| **Education** | | | |
| Primary | 19 | 2 (33.3) | 17 (39.5) |
| Secondary | 22 | 2 (33.3) | 20 (46.5) |
| Tertiary | 8 | 2 (33.3) | 6 (14.0) |
| **Body mass index (BMI)** | | | |
| <17.5 | 4 | 3 (75.0) | 1 (25.0) |
| 17.5 - <20.0 | 10 | 8 (80.0) | 2 (20.0) |
| 20.0 - <22.5 | 17 | 15 (88.2) | 2 (11.8) |
| 22.5 - <25.0 | 5 | 5 (100.0) | 0 (0.0) |
| 25.0 - <27.5 | 5 | 4 (80.0) | 1 (20.0) |
| 27.5 - <30.0 | 3 | 3 (100.0) | 0 (0.0) |
| ≥30.0 | 5 | 5 (100.0) | 0 (0.0) |
| **Risk factors for colonisation** | | | |
| **Smoking** | | | |
| Yes | 13 | 13 (100.0) | 0 (0.0) |
| No | 36 | 30 (83.3) | 6 (16.7) |
| **Diabetes** | | | |
| Yes | 1 | 1 (100.0) | 0 (0.0) |
| No | 48 | 42 (87.5) | 6 (12.5) |
| **HIV** | | | |
| Yes | 2 | 1 (50.0) | 1 (50.0) |
| No | 47 | 42 (89.4) | 5 (10.6) |
| **Admitted to a healthcare facility in the past one year** | | | |
| Yes | 5 | 5 (100.0) | 0 (0.0) |
| No | 44 | 38 (86.4) | 3 (13.6) |
| **Used any antibiotics in the past 3 months** | | | |
| Yes | 20 | 16 (80.0) | 4 (20.0) |
| No | 29 | 27 (93.1) | 2 (6.9) |
| **Skin or soft tissue infections in the past one year** | | | |
| Yes | 3 | 2 (66.7) | 1 (33.3) |
| No | 46 | 41 (89.1) | 5 (10.9) |
| **Previously admitted to ICU** | | | |
| Yes | 0 | (0.0) | 0 (0.0) |
| No | 49 | 43 (87.8) | 6 (12.2) |

**Table 2.** Contd.

| | | | |
|---|---|---|---|
| **Open wound** | | | |
| Yes | 18 | 16 (88.9) | 2 (11.1) |
| No | 31 | 27 (87.1) | 4 (12.9) |
| **Outpatient clinic in the past one year** | | | |
| Yes | 12 | 8 (66.7) | 4 (33.3) |
| No | 37 | 35 (94.6) | 2 (5.4) |
| **Household members** | | | |
| ≤4 | 37 | 32 (86.5) | 5 (13.5) |
| >4 | 12 | 11 (91.7) | 1 (8.3) |
| **Soft tissue infection** | | | |
| Yes | 3 | 2 (66.7) | 1 (33.3) |
| No | 46 | 41 (89.1) | 5 (10.9) |

in the past 3 months. None of the patients colonised by MRSA had been admitted in the past year (Table 2).

### Risk factors associated with *S. aureus* colonisation

Univariate and multivariate analysis of risk factors for nasal carriage was done. Statistically significant findings were found on multivariate analysis as pertains to BMI whereby patients who were underweight (BMI <18.5) were less likely to be colonised than obese patients (BMI ≥ 30) (OR 0.2 [95% CI 0.0-0.9]). None of the other risk factors for colonization were found to be significant in the study.

Male patients were slightly more likely to be carriers of *S. aureus* than female patients (OR 1.2 [95% CI 0.5-2.9]). Non-smokers were less likely to be carriers when compared with smokers (OR 0.7 [95% CI 0.7-3.0]). Persons from households with less than 4 persons were also more likely to be colonised compared with persons from houses with more than 4 persons (OR 1.3 [95% CI 0.6 - 2.8]).

In contrast to currently available literature, patients who were HIV positive, diabetic, had open wounds or soft tissue infections were less likely to be colonised; however, these findings did not reach statistical significance.

Patients who had no history of antibiotic use in the prior 3 months or had not visited an outpatient facility in the past year were less likely to be colonised (OR 0.8). There was no effect of prior admission to a healthcare facility (OR 1). Table 3 summarises the findings.

## DISCUSSION

### Prevalence of *S. aureus* colonisation

The overall prevalence of colonisation by S. *aureus* was 24.7% whereas the MRSA prevalence was 3.03%. These findings are in line with those of Kluytmans et al. (1997) who did a review of *S. aureus* carriage and found a mean carriage rate of 35.7% among patients on admission with a wide range of 10.2 to 85.0%.

The carriage rate is higher compared to that found in some previous studies. Aiken et al. (2014) reported a 10.1% carriage rate when screening inpatients in Kenya while Nelwan et al. (2018) in Indonesia reported a 15.6% carriage rate when screening elective surgery patients whereas Egyir et al. (2018) found a 17% carriage rate among inpatients in paediatric and surgical wards in Ghana. The differences may be explained by the fact that Aiken et al. (2014) used a different sampling technique in which he did repeated ward surveys of the inpatients, whereas Egyir et al. (2018) included paediatric patients in their study which differs from the present study in which we only recruited adult patients.

Joachim et al. (2017) in Tanzania found a higher overall nasal carriage rate of 34.5% whereas Kolawole et al. (2013) in Nigeria reported a higher carriage rate of 31.8%. The higher prevalence reported by Joachim et al. (2017) could be due to the collection of a second swab 48 to 72 h after admission whereas Kolawole et al. (2013) utilised Polymerase Chain Reaction (PCR) to identify carriers. Both methods have been shown to increase detection rates of carrier status.

The overall MRSA prevalence matches that of Troillet et al. (1998) who found a carriage rate of 2.6% when nasal swabs were taken and 3.1% when nasal and wound swabs were taken for culture and sensitivity at admission.

Aiken et al. (2014) reported a higher proportion of MRSA at 6.9% when compared with the 3.03% that we got in the current study. The difference may be explained by the fact that Aiken et al. (2014) used a different sampling technique in which he did repeated ward surveys of the inpatients. On the other hand, Joachim et

**Table 3.** Univariate and multivariate association between colonisation and risk factors.

| Characteristic | N=198 | Colonization [n (%)] | Univariate p-value; OR (95% CI) | Multivariate p-value; OR (95% CI) |
|---|---|---|---|---|
| **Age** | | | | |
| 18-30 | 82 | 19 (23.2) | 0.594; 1.8 (0.2 -16.0) | 0.909; 1.2 (0.1 -12.7) |
| 31-60 | 109 | 29 (26.6) | 0.481; 2.2 (0.3-18.8) | 0.785; 1.4 (0.1 -14.3) |
| >60 | 7 | 1 (14.3) | 1.0 | 1.0 |
| **Sex** | | | | |
| Male | 167 | 42 (25.1) | 0.761; 1.2 (0.5-2.9) | 0.711; 1.2 (0.4 -3.8) |
| Female | 31 | 7 (22.6) | 1.0 | 1.0 |
| **BMI** | | | | |
| <18.5 | 39 | 6 (15.4) | 0.087; 0.3 (0.1 -1.2) | 0.037; 0.2 (0.0 -0.9) |
| 18.5-24.9 | 118 | 30 (25.4) | 0.319; 0.5 (0.2 -1.8) | 0.150; 0.3 (0.1 -1.5) |
| 25-29.9 | 28 | 8 (28.6) | 0.528; 0.6 (0.2 -2.6) | 0.312; 0.4 (0.1 -2.2) |
| >=30 | 13 | 5 (38.5) | 1.0 | 1.0 |
| **Household Members** | | | | |
| ≤4 | 141 | 37 (26.2) | 0.445; 1.3 (0.6-2.8) | 0.241; 1.6 (0.7 -3.6) |
| >4 | 57 | 12 (21.1) | 1.0 | 1.0 |
| **Diabetes** | | | | |
| Yes | 8 | 1 (12.5) | 1.0 | 1.0 |
| No | 190 | 48 (25.3) | 0.426; 2.4 (0.3 -19.7) | 0.358; 3.3 (0.3 -41.5) |
| **HIV** | | | | |
| Positive | 12 | 2 (16.7) | 1.0 | 1.0 |
| Negative | 186 | 47 (25.3) | 0.508; 1.7 (0.4 -8.0) | 0.213; 3.2 (0.5 -20.3) |
| **Open wound** | | | | |
| Yes | 75 | 18 (24.0) | 1.0 | 1.0 |
| No | 123 | 31 (25.2) | 0.849; 1.1 (0.5 -2.1) | 0.678; 1.2 (0.6 -2.4) |
| **Soft tissues infection** | | | | |
| Yes | 19 | 3 (15.8) | 1.0 | 1.0 |
| No | 179 | 46 (25.7) | 0.481; 1.6 (0.4 -5.8) | 0.406; 1.8 (0.4 -7.5) |
| **Smoking** | | | | |
| Yes | 43 | 13 (30.2) | 1.0 | 1.0 |
| No | 155 | 36 (23.3) | 0.348; 0.7 (0.3 -1.5) | 0.381; 0.7 (0.3 -1.6) |
| **Education** | | | | |
| Primary | 71 | 19 (26.8) | 0.92; 1.1 (0.4 -2.7) | 0.849; 1.1 (0.4 -3.1) |
| Secondary | 96 | 22 (22.9) | 0.742; 0.9 (0.3 -2.2) | 0.723; 0.8 (0.3 -2.3) |
| Tertiary | 31 | 8 (25.8) | 1.0 | 1.0 |
| **Admitted to a healthcare facility in the past 1 year** | | | | |
| Yes | 20 | 5 (25.0) | 1.0 | 1.0 |
| No | 178 | 44 (24.7) | 0.978; 1.0 (0.3 -2.9) | 0.948; 1 (0.3 -3.5) |
| **Used any antibiotics in the past 3 months** | | | | |
| Yes | 75 | 20 (26.7) | 1.0 | 1.0 |
| No | 123 | 29 (23.6) | 0.625; 0.8 (0.4 -1.6) | 0.405; 0.7 (0.3 -1.6) |

**Table 3.** Contd.

| | | | | |
|---|---|---|---|---|
| **Outpatient clinic in the past 1 year** | | | | |
| Yes | 44 | 12 (27.3) | 1.0 | 1.0 |
| No | 154 | 37 (24.0) | 0.660; 0.8 (0.4 -1.8) | 0.633; 0.8 (0.3 -2.1) |
| **Previously admitted to ICU** | | | | |
| Yes | 1 | 0 (0.0) | 1.0 | 1.0 |
| No | 197 | 49 (24.9) | - | - |

al. (2017) in Tanzania found a MRSA carriage rate of 8.5%. The difference in MRSA carriage rates could be explained by the different populations studied, Joachim et al. (2017) was studying prevalence at admission among medical patients who are more likely to be chronically ill and therefore more likely to be colonised by MRSA as a result of repeated antibiotic exposure and visits to healthcare facilities which are both risk factors for colonisation by MRSA.

Egyir et al. (2018) in Ghana found a MRSA prevalence of 3.6%. The difference in results may be explained by the different populations studied as Egyir et al. included paediatric patients.

Kolawole et al. (2013) in Nigeria reported MRSA prevalence of 3.7% when testing patients on admission to surgical wards. The higher prevalence may be explained by the fact that nasal and cutaneous sites were tested on admission and extra nasal testing has been shown to increase detection rates of the carrier status.

While the MRSA prevalence in this study is 3.03% on admission there remains the possibility of spread of MRSA within the ward to other patients especially with the overcrowding, bed sharing, understaffing and poor infection control adherence sometimes witnessed in orthopaedic wards. Clements et al. (2018) showed that overcrowding and understaffing led to failure to control MRSA leading to increased hospital stay, bed blocking hence worsening overcrowding and leading to a vicious cycle characterized by further infection control failure.

### Risk factors associated with colonisation by *S. aureus*

Statistically significant findings were found on multivariate analysis as pertains to BMI whereby patients who were underweight (BMI <18.5) were less likely to be colonised than obese patients (BMI ≥ 30) (OR 0.2 [95% CI 0.0-0.9]).

The other risk factors for colonisation by MSSA and MRSA did not reach significance. Joachim et al in a similar study in Tanzania with 258 patients also found no association between risk factors and colonisation. Smoking was found to predispose to colonisation contrary to results by Sivaraman et al. (2009). Diabetics, HIV positive patients, patients with soft tissue infections and patients with open wounds were found to be less likely to be colonised contrary to what is reported in the literature (Troillet et al., 1998; Immergluck et al., 2017; Amir et al., 1995). Patients who had no history of antibiotic use in the prior 3 months or had not visited an outpatient facility in the past year were less likely to be colonised. This and the lack of significance of other findings in this study may be due to the smaller sample size and unique characteristics of the cohort, that is, predominantly young adult men with no comorbidities. This may also be due to poor recall on the part of the patients e.g. majority of respondents who said they had antibiotics prior to admission could not tell us which antibiotic they had been using. Additionally, some of the risk factors such as diabetes, HIV, prior admission to ICU, admission to a nursing home or taking care of a bedridden patient had a small number of patients and in some cases no patients responding positively possibly contributing to the lack of association as some of these are important risk factors for colonisation by MSSA and MRSA.

## Conclusion

The prevalence of colonisation by *S. aureus* is high amongst patients being admitted to the orthopaedic wards at KNH when compared with previous local studies and amongst these are patients who are already colonised by MRSA.

## Recommendations

(1) Routine decolonisation of orthopaedic patients prior to surgery with intranasal mupirocin or nasal povidone iodine and chlorhexidine body washes.
(2) Need for more research to determine if those with MSSA and MRSA have higher infection rates.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors acknowledged the staff of the Paediatrics Laboratory, University of Nairobi.

### REFERENCES

Aiken AM, Mutuku IM, Sabat AJ, Akkerboom V, Mwangi J, Scott JAG (2014). Carriage of *Staphylococcus aureus* in Thika Level 5 Hospital, Kenya: A cross-sectional study. Antimicrobial Resistance and Infection Control 3(1):22.

Amir M, Paul J, Batchelor B, Kariuki S, Ojoo J, Waiyaki P (1995). Nasopharyngeal carriage of *Staphylococcus aureus* and carriage of tetracycline-resistant strains associated with HIV-seropositivity. European Journal of Clinical Microbiology and Infectious Diseases 14(1):34-40.

Chen A, Rao N, Wessel C (2013). *Staphylococcus aureus* Screening and Decolonization in Orthopaedic Surgery and Reduction of Surgical Site Infections. Clinical Orthopedics and Related Research 471:2383-2399.

Clements A, Halton K, Graves N, Pettitt A, Morton A, Looke D (2008). Overcrowding and understaffing in modern health-care systems: key determinants in meticillin-resistant *Staphylococcus aureus* transmission. The Lancet Infectious Diseases 8(7):427-434.

Egyir B, Larsen AR, Addo K, Newmann M, Guardabassi L (2018). Nasal carriage of *staphylococcus aureus* and methicillin resistant *staphylococcus aureus* (MRSA) among health workers and inpatients at korle bu teaching hospital in Ghana. American Journal of Tropical Medicine and Hygiene 87(5):25.

Immergluck L, Jain S, Ray S, Mayberry R, Satola S, Parker T (2017). Risk of Skin and Soft Tissue Infections among Children Found to be *Staphylococcus aureus* MRSA USA300 Carriers. Western Journal of Emergency Medicine 18(2):201.

Jeans E, Holleyman R, Tate D, Reed M, Malviya A (2018). Methicillin sensitive *staphylococcus aureus* screening and decolonisation in elective hip and knee arthroplasty. Journal of Infection 77(5):405-409.

Joachim A, Moyo SJ, Nkinda L, Majigo M, Mbaga E, Mbembati N (2017). Prevalence of methicillin-resistant *Staphylococcus aureus* carriage on admission among patients attending regional hospitals in Dar es Salaam, Tanzania. BMC Research Notes 10(1):1-7.

Kalmeijer MD, Nieuwland-Bollen EV, Bogaers-Hofman D, Baere GAJ de, Kluytmans JAJW (2000). Nasal Carriage of *Staphylococcus aureus*: Is a Major Risk Factor for Surgical-Site Infections in Orthopedic Surgery. Infection Control and Hospital Epidemiology 21(05):319-323.

Kluytmans J, van Belkum A, Verbrugh H (1997). Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clinical Microbiology Reviews 10(3):505-520.

Kolawole DO, Adeyanju A, Schaumburg F, Akinyoola AL, Lawal OO, Amusa YB (2013). Characterization of Colonizing *Staphylococcus aureus* Isolated from Surgical Wards' Patients in a Nigerian University Hospital. PLoS One 8(7).

Krejcie R, Morgan D (1970). Determining Sample Size for Research Activities. Educational and Psychological Measurement 30(3):607-610.

Nelwan EJ, Sinto R, Subekti D, Adiwinata R, Waslia L, Loho T (2018). Screening of methicillin-resistant *Staphylococcus aureus* nasal colonization among elective surgery patients in referral hospital in Indonesia. BMC Research Notes 11(1):1-5.

Ning J, Wang J, Zhang S, Sha X (2020). Nasal colonization of *Staphylococcus aureus* and the risk of surgical site infection after spine surgery: a meta-analysis. The Spine Journal 20(3):448-456.

Reighard A, Diekema D, Wibbenmeyer L, Ward M, Herwaldt L (2009). *Staphylococcus aureus* Nasal Colonization and Colonization or Infection at Other Body Sites in Patients on a Burn Trauma Unit. Infection Control and Hospital Epidemiology 30(08):721-726.

Schmidt A, Benard S, Cyr S (2015). Hospital Cost of Staphylococcal Infection after Cardiothoracic or Orthopedic Operations in France: A Retrospective Database Analysis. Surgical Infections 16(4):428-435.

Sivaraman K, Venkataraman N, Cole AM (2009). *Staphylococcus aureus* nasal carriage and its contributing factors. Future Microbiology 4(8):999-1008.

Troillet N, Carmeli Y, Samore MH, Dakos J, Eichelberger K, DeGirolami PC (1998). Carriage of methicillin-resistant *Staphylococcus aureus* at hospital admission. Infection Control and Hospital Epidemiology 19(3):181-185.

US Centers for Disease Control and Prevention (2011). Specimen collection guidelines. https://www.cdc.gov/urdo/downloads/speccollectionguidelines.pdf.

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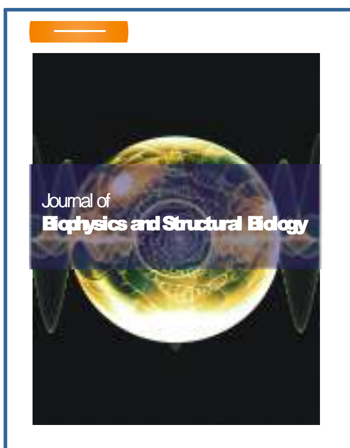

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