OPEN ACCESS

December 2023
ISSN 1996-0808
DOI: 10.5897/AJMR
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

About AJMR

The African Journal of Microbiology Research (AJMR) is a peer reviewed open access journal. The journal commenced publication in May 2007. The journal covers all areas of microbiology such as environmental microbiology, clinical microbiology, immunology, virology, bacteriology, phycology, molecular and cellular biology, molecular microbiology, food microbiology, mycology and parasitology, microbial ecology, probiotics and prebiotics and industrial microbiology.

Indexing

CAB Abstracts, CABI's Global Health Database, Chemical Abstracts (CAS Source Index)
Dimensions Database, Google Scholar, Matrix of Information for The Analysis of Journals (MIAR),
Microsoft Academic, Research Gate

Open Access Policy

Open Access is a publication model that enables the dissemination of research articles to the global community without restriction through the internet. All articles published under open access can be accessed by anyone with internet connection.

The African Journal of Microbiology Research is an Open Access journal. Abstracts and full texts of all articles published in this journal are freely accessible to everyone immediately after publication without any form of restriction.

Article License

All articles published by African Journal of Microbiology Research are licensed under the Creative Commons Attribution 4.0 International License. This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited. Citation should include the article DOI. The article license is displayed on the abstract page the following statement:

This article is published under the terms of the Creative Commons Attribution License 4.0
Please refer to https://creativecommons.org/licenses/by/4.0/legalcode for details about Creative Commons Attribution License 4.0

Article Copyright

When an article is published by in the African Journal of Microbiology Research, the author(s) of the article retain the copyright of article. Author(s) may republish the article as part of a book or other materials. When reusing a published article, author(s) should; Cite the original source of the publication when reusing the article. i.e. cite that the article was originally published in the African Journal of Microbiology Research. Include the article DOI, Accept that the article remains published by the African Journal of Microbiology Research (except in occasion of a retraction of the article).

The article is licensed under the Creative Commons Attribution 4.0 International License.

A copyright statement is stated in the abstract page of each article. The following statement is an example of a copyright statement on an abstract page.
Copyright ©2016 Author(s) retains the copyright of this article.

Self-Archiving Policy

The African Journal of Microbiology Research is a RoMEO green journal. This permits authors to archive any version of their article they find most suitable, including the published version on their institutional repository and any other suitable website.

Digital Archiving Policy

The African Journal of Microbiology Research is committed to the long-term preservation of its content. All articles published by the journal are preserved by Portico. In addition, the journal encourages authors to archive the published version of their articles on their institutional repositories and as well as other appropriate websites.
https://www.portico.org/publishers/ajournals/

Metadata Harvesting

The African Journal of Microbiology Research encourages metadata harvesting of all its content. The journal fully supports and implement the OAI version 2.0, which comes in a standard XML format. See Harvesting Parameter

Memberships and Standards

Academic Journals strongly supports the Open Access initiative. Abstracts and full texts of all articles published by Academic Journals are freely accessible to everyone immediately after publication.

All articles published by Academic Journals are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited.

Crossref is an association of scholarly publishers that developed Digital Object Identification (DOI) system for the unique identification published materials. Academic Journals is a member of Crossref and uses the DOI system. All articles published by Academic Journals are issued DOI.

Similarity Check powered by iThenticate is an initiative started by CrossRef to help its members actively engage in efforts to prevent scholarly and professional plagiarism. Academic Journals is a member of Similarity Check.

CrossRef Cited-by Linking (formerly Forward Linking) is a service that allows you to discover how your publications are being cited and to incorporate that information into your online publication platform. Academic Journals is a member of CrossRef Cited-by.

Academic Journals is a member of the International Digital Publishing Forum (IDPF). The IDPF is the global trade and standards organization dedicated to the development and promotion of electronic publishing and content consumption.

Contact

Editorial Office: ajmr@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJMR

Submit manuscript online http://ms.academicjournals.org

Academic Journals
73023 Victoria Island, Lagos, Nigeria
ICEA Building, 17th Floor,
Kenyatta Avenue, Nairobi, Kenya.

Editors

Prof. Adriano Gomes da Cruz
University of Campinas (UNICAMP),
Brazil.

Prof. Ashok Kumar
School of Biotechnology
Banaras Hindu UniversityUttar Pradesh,
India.

Dr. Mohd Fuat Abd Razak
Infectious Disease Research Centre,
Institute for Medical Research, Jalan
Pahang, Malaysia.

Dr. Adibe Maxwell Ogochukwu
Department of Clinical Pharmacy and
Pharmacy Management,
University of Nigeria
Nsukka, Nigeria.

Dr. Nadezhda Fursova
Molecular Microbiology,
State Research Center for Applied
Microbiology and Biotechnology,
Russia.

Dr. Mehdi Azami
Parasitology & Mycology Department
Baghaeei Lab.
Isfahan, Iran.

Dr. Franco Mutinelli
Istituto Zooprofilattico Sperimentale delle
Venezie Italy.

Prof. Ebiamadon Andi Brisibe
University of Calabar,
Calabar,
Nigeria.

Prof. Nazime Mercan Dogan
Department of Biology
Faculty of Science and Arts
University Denizli Turkey.

Prof. Long-Liu Lin
Department of Applied Chemistry
National Chiayi University
Chiayi County Taiwan.

Prof. Natasha Potgieter
University of Venda
South Africa.

Dr. Tamer Edirne
Department of Family Medicine
University of Pamukkale
Turkey.

Dr. Kwabena Ofori-Kwakye
Department of Pharmaceutics
Kwame Nkrumah University of Science &
Technology
Kumasi, Ghana.

Dr. Tülin Askun
Department of Biology
Faculty of Sciences & Arts
Balikesir University Turkey.

Dr. James Stefan Rokem
Department of Microbiology & Molecular
Genetics
Institute of Medical Research Israel – Canada
The Hebrew University – Hadassah Medical
School Jerusalem, Israel.

Editors

Dr. Afework Kassu
University of Gondar
Ethiopia.

Dr. Wael Elnaggar
Faculty of Pharmacy
Northern Border University
Rafha Saudi Arabia.

Dr. Maulin Shah
Industrial Waste Water Research Laboratory
Division of Applied & Environmental Microbiology, Enviro Technology Limited
Gujarat, India.

Dr. Ahmed Mohammed
Pathological Analysis Department
Thi-Qar University College of Science
Iraq.

Prof. Naziha Hassanein
Department of Microbiology
Faculty of Science
Ain Shams University
Egypt.

Dr. Shikha Thakur
Department of Microbiology
Sai Institute of Paramedical and Allied Sciences India.

Prof. Pongsak Rattanachaikunsopon
Department of Biological Science,
Ubon Ratchathani University,
Thailand.

Dr. Rafael Lopes e Oliveira
Chemical Engineering,
Amazon State University - Uea,
Brazil.

Dr. Annalisa Serio
Faculty of Bioscience and Technology for Food, Agriculture and Environment,
University of Teramo.
Italy

Dr. Samuel K Ameyaw
Civista Medical Center
USA.

Dr. Mahmoud A. M. Mohammed
Department of Food Hygiene and Control
Faculty of Veterinary Medicine
Mansoura University Egypt.

Dr. Anubrata Ghosal
Department of Biology
MIT - Massachusetts Institute of Technology
USA.

Dr. Bellamkonda Ramesh
Department of Food Technology
Vikrama Simhapuri University
India.

Dr. Sabiha Yusuf Essack
Department of Pharmaceutical Sciences
University of KwaZulu-Natal
South Africa.

Dr. Navneet Rai
Genome Center
University of California Davis USA.

Dr. Iheanyi Omezuruike Okonko
Department of Virology
Faculty of Basic Medical Sciences
University of Ibadan
Ibadan, Nigeria.

Dr. Mike Agenbag
Municipal Health Services,
Joe Gqabi,
South Africa.

Dr. Abdel-Hady El-Gilany
Department of Public Health & Community Medicine, Faculty of Medicine
Mansoura University
Egypt.

Dr. Bachir Raho Ghalem
Biology Department,
Faculty of natural sciences and life,
Mascara university,
Algeria.

Table of Content

The assessment of contamination level of multidrug resistant bacteria and antibiotic residue in chicken manure 285

Romuald Christ Kipré, Bernadette Gblossi Goualie, Monique Aiza Ainyakou-Sanga, Daniel Kouamé Kra and Germain Alfred Karou

Molecular identification of biofilm-producing *Bacillus* species and yeasts isolated from food sources and their interaction with *Lysinibacillus louembei* strain 290

Moïse Doria Kaya-Ongoto, Christian Aimé Kayath, Nelly Josiane Awah-Lekaka Niebi and Etienne Nguimbi

Prevalence, antibiotic resistance of Staphylococcus aureus isolated from layer farms (eggs, droppings/litter) in the outskirts of the city of Ouagadougou, Burkina Faso 306

Abdallah Sawadogo, Assèta Kagambèga, Daniel Soro, Henseni Bognini, Michel Dione, Haoua Cissé, Ibonyé Dieni and Nicolas Barro

Acute bacterial meningitis ecosystem in Cote D'ivoire from 2012 to 2020 319

Sophia Chiayé Okpo, Clarisse Kouamé Elogne, Tatiana Asseh Kangah, Jean Claude Blessa Anné, Kouadio N'guessan, Solange Kakou-Ngazoa and Mireille Dosso

Vol. 17(12), pp. 285-290, December 2023
DOI: 10.5897/AJMR2023.9717
Article Number: E787EB771610
ISSN: 1996-0808
Copyright©2023
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

The assessment of contamination level of multidrug resistant bacteria and antibiotic residue in chicken manure

Romuald Christ Kipré[1], Bernadette Gblossi Goualie[1]*, Monique Aiza Ainyakou-Sanga[1], Daniel Kouamé Kra[2] and Germain Alfred Karou[1]

[1]UFR Biosciences, Félix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Ivory Coast.
[2]UFR Natural Sciences, Nangui ABROGOUA University, 02 BP 801 Abidjan 02, Ivory Coast.

Received 1 September, 2023; Accepted 21 November, 2023

This study aimed to assess the contamination level of chicken litter by antibiotic residues and multidrug resistant (MDR) bacteria. A total of 28 chicken litter samples were aseptically collected in Abidjan poultry farms in December 2021. The bacteria including *Salmonella, Escherichia coli* and *Staphyloccocus aureus* were isolated on specific culture media and were identified using biochemical tests. Antibiotics susceptibility test of the isolates was performed by the disk diffusion method. Finally, the antibiotic residues were detected in litter samples by HPLC. Among the analyzed samples, 21 were positive for the three bacteria. Moreover, *S. aureus* isolates were resistant to tetracycline, clindamycine, trimethoprime, and erythromycine while *E. coli* and *Salmonella* strains were resistant to minocycline, nalidixic acid, and tetracycline with resistance rate above 75% for all strains. The MDR including 3 to 6 antibiotic classes were found in 90 and 23.07% of *S. aureus* and *E. coli* strains, respectively. Moreover, chicken litter samples were contaminated by antibiotic residues mainly ciprofloxacine, erythromycine, spiramycine and oxytétracycline with amounts between 0.05 ± 0.002 and 8.41 ± 2.03 mg/kg. These results showed the need to treat chicken litter before being used as fertilizer to reduce their negative impact on environment and health.

Key words: Chicken litter, multidrug resistant bacteria, antibiotic residues, soil, crops pollution.

INTRODUCTION

In Côte d'Ivoire, livestock production and agricultural subsistence farming practices have intensified in recent decades. The poultry sector specifically, has expanded since 2006 when restrictions on import of frozen chicken were introduced (ANADER, 2018). Thus, the annual production had risen from 20,000 tons/year in 2011 to 174,000 tons/year in 2020 (IPRAVI, 2021). With this expansion of the poultry industry in the country, production of poultry litter as a waste product has also increased, further encouraging its use as manure for agriculture system.

In general, this manure is used as fertilizer by many farmers but without prior treatment. In the region of Abidjan in particular, poultry litter is mostly used by

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

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

market gardeners. Indeed, even if farmers have easy access to groundwater for crop irrigation (through spraying water method), they are limited by the low fertility of the coastal sandy soils of Abidjan area. Thus, to satisfy growing urban demand and to improve crop productivity, they have adopted intensive practices, such as application of high rates of poultry manure as fertilizers, which is available locally.

However, besides its rich organic content, poultry litter and manure can be contaminated with various types of pathogens including viruses, bacteria, parasites, and fungi. Indeed, foodborne bacteria such as *Escherichia coli, Salmonella, Staphyloccocus aureus* and *Campylobacter* species have been isolated in poultry intestinal tract and in litter (Viegas et al., 2012).

These bacteria pose a risk of transmission to humans, animals and the environment; especially considering their ability to survive for months in water, soil and crops (Merchant et al., 2012; Oliveira et al., 2012). Moreover, in developing countries as Côte d'Ivoire, there is no surveillance system for antibiotics use during breeding. Thus, besides the risk of microbial contamination, there is an additional concern of transmission of multidrug resistant (MDR) bacteria, due to this reported high use of these antibiotics either as growth promoters or for prophylactic purposes (Dahshan et al., 2015; Goualié et al., 2020; Roth et al., 2018). However, so far, little is known of the microbiological and chemical components of poultry litter used as fertilizer in Côte d'Ivoire.

This study therefore aim to assess the level of contamination in chicken litter use as manure in vegetable culture in Abidjan of antibiotic residues and MDR bacteria in view to evaluate the risks associated to the use of this litter without prior treatment.

MATERIALS AND METHODS

Chicken litter samples collection

A total of 28 litters samples were randomly collected from 16 farms in five municipalities including Yopougon, Songon, Bingerville, and Anyama because of their high poultry production capacity in the District of Abidjan, Côte d'Ivoire. In each farm, the litter was aseptically samplied directely from previously stored bags. Each collected sample was packed in sterile plastic bags and transported to the Biotechnology laboratory of the Félix Houphouët-Boigny University. Among these 28 litters samples, 6 were from Yopougon, 5 were collected in Songon, 9 were from Bingerville and 8 were from Anyama.

Microbiological analyses of the chicken litter

For each sample, 10 g of poultry litter was transferred in 90 ml of Buffered Peptone Water (BPW) (Bio-Rad Marne-la-Coquette, France). The suspension was homogenized before a serial dilution in salt buffer. Then, the enumeration of *E. coli* and *S. aureus* was performed by culture, respectively, on TBX agar (Conda, Madrid, Spain) as described in ISO/TS 16649-3 guidelines and according to the directives of AFNOR V 08- 057-1: 2004 onto Baird-Parker (BP) agar medium (CM 275, Oxoid, UK) supplemented with egg yolk tellurite emulsion (SR 54, Oxoid, UK).

Thus, for each dilution (10^{-1} to 10^{-5}), 0.1 ml was spread onto the specific medium and all plates were incubated at 37°C during 24 h.

Concerning *Salmonella*, the method described by ISO 6579 was used. Thus, the BPW was directly incubated during 18 h at 37°C as pre-enrichment medium. From each pre-enriched sample, 0.1 ml was used to inoculate 10 ml of Rappaport-Vassiliadis broth (Bio-Rad, Marne-la-Coquette, France). Samples were incubated during 19 h at 42°C and *Salmonella* isolation was achieved on Hektoen agar (Bio-Rad, Marne-la-Coquette, France) medium at 37°C for 24 h. After incubation, one typical colony was selected on each medium for morphological (Gram staining) and biochemical identification by using API®20E gallery (BIOMERIEUX, France) for *E. coli* and *Salmonella* strains and API® Staph gallery for *S. aureus* isolates. The isolates then were stored at -20°C in liquid medium supplemented with glycerol (25%).

Antibiotics sensitivity test of *E. coli*, *Salmonella* and *S. aureus* isolated from chicken litter

Antibiotic susceptibility tests were carried out on 31 *E. coli*, *S. aureus* and *Salmonella* strains by using the Kirby-Bauer (Bauer et al., 1966) diffusion method in the Mueller-Hinton agar medium. The reference strain notably *E. coli* ATCC 25922 was used as a quality control for the susceptibility testing. The following antibiotics were tested for *E. coli* and *Salmonella*: ampicillin (10 µg), cefalotine (30 µg), cefepime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), imipenem (10 µg), tetracycline (30 µg), minocycline (30 µg), gentamicine (10 µg), amikacine (30 µg), nalidixic acid (30 µg), norfloxacine (5 µg), ciprofloxacine (5 µg), chloramphenicol (30 µg), and trimethoprime (25 µg). For *S. aureus*, penicilline (1 IU), tobramycine (10 µg), gentamicine (10 µg), minocycline (30 µg), tetracycline (30 µg), erythromycine (15 µg), clindamycine (2 µg), trimethoprime (25 µg), fusidic acid (10 µg), vancomycine (30 µg), rifampicine (5 µg), norfloxacine (10 µg), fosfomycine (200 µg), kanamycine (30 µg), and cefoxitine (30 µg) were tested. All commercial antibiotic dics were purchased from BioRad (France). The inoculated plates were incubated during 24 h at 37°C and the inhibition zones were measured and analyzed according to the CASFM/EUCAST (2022) guidelines.

Antibiotics residues detection in chicken litter

Before the determination of the antibiotic residues in the chicken litter, the samples collected in each commune were mixed to make four large samples. From each large sample, 10 g was collected three times and analyzed separately. Thus, twelve (12) representative antibiotics which were widely used in poultry production system (Goualie et al., 2020) were targeted. Each drug was extracted by ultrasonic method for the classes of macrolides and betalactamin or by using a chemical solvent for sulfonamides, tetracyclines, and fluoroquinolones. Then, the target antibiotics were subsequently analyzed using a high performance liquid chromatography (HPLC). The HPLC Equipment (Waters, USA) consists of the UV visible spectrophotometer (Packard), the systems using a Waters 986 Tunable absorbance detector, a Waters 600 controller, an X-Act degasser, a Waters 717 plusautosampler, a Genesis C18 column (4.6 × 150 mm, 4 µm), a C18 guard column (10 × 4 mm, 4 µm) and a column support (10 mm). For each antibiotic, the standard consisted of a pure solution of the target antibiotic (Sigma-Aldrich). Each test was repeated three times and results were expressed as mean ± standard deviation.

Table 1. Antibiotic resistance profile of *S. aureus* strains.

Antibiotics used	Resistance % (number of strains)	Number of antibiotic classes
K	20 (2/10)	1
E	90 (9/10)	1
CIP	10 (1/10)	1
CMN	80 (8/10)	1
TMP	90 (9/10)	1
VA	30 (3/10)	1
CHL	10 (1/10)	1
TET	90 (9/10)	1
TMP/VA	30 (3/10)	2
TE/CHL/VA/E	10 (1/10)	4
TMP/ TET /CMN/E	30 (3/10)	4
TMP/ TET /CMN/E/K	10 (1/10)	5
TMP/ TET/CMN/E/VA	20 (2/10)	5
TMP/ TET /CMN/E/CIP	10 (1/10)	5
TMP/ TET /CMN/E/VA/K	10 (1/10)	6

K: Kanamycine; E: erythromycine; CIP: ciprofloxacine; CMN: clindamycine; TMP: trimethoprime; VA: vancomycine; CHL: chloramphenicol; TET: tetracycline.

RESULTS

Prevalence and antibiotic profile of the bacteria isolated from chicken litter

A total of 28 poultry litter samples were collected and analyzed for *E. coli, S. aureus*, and *Salmonella* isolation. All tested samples (100%) were positive for *E. coli* and *S. aureus,* while 21 (75%) samples were positive for *Salmonella*. Among these samples positive for *Salmonella* genus, 4 were from Yopougon, 5 were from Songon, 8 were from Bingerville, and 4 were from Anyama.

The bacterial load for *E. coli* was between 2.60×10^2 and $3,8\times10^2$ CFU/g, while it was 5.2×10^3 and 9.84×10^4 CFU/g for *S. aureus*. Among the 77 bacteria strains (one confirmed strain per each positive sample), 31 strains including 8 *Salmonella*, 13 *E. coli* and 10 *S. aureus* were selected for the antibiotics susceptibility testing. The frequency of antibiotic resistance for tested strains are shown in Tables 1 to 3. Results show that *S. aureus* strains were found with high resistance to tetracycline clindamycine, erythromycine and trimethoprime with rate above 80% (Table 1). Comparatively to these drugs, low resistance was observed with kanamycine (20%), ciprofloxacine (10%), vancomycine (30%), and chloramphenicol (10%). While, no resistance was observed for gentamycine, tobramycine, fusidic acid and cefoxitine. However, cross resistance to sulfonamides (trimethoprime) and glycopeptides (vancomycine) was observed in 30% of *S. aureus* tested strains. In addition, the Multi-Drug Resistance (MDR) including four, five and six antibiotics families was detected in 9 (90%) of *S. aureus* strains (Table 1).

Among the *E. coli*, 10 (76.92%) were resistant to tetracycline and minocycline belonging to tetracyclines classes and to nalidixic acid (fluoroquinolones) (Table 2). While, low resistance was found against ampiciline (7.69%), gentamycine (15.38%) and to ciprofloxacine (7.69%). Furthermore, the MDR including three antibiotics classes was detected in 3 (23.07%) *E. coli* isolates (Table 2). Among the 15 drugs tested in this work, 6 were efficient against *E. coli* with sensitive rate at 100%.

Concerning the 8 *Salmonella* strains, seven were resistant to one or more drugs tested in this study. The highest percentages of antimicrobial resistance were found for nalidixic acid (87.5%), tetracycline (87.5%) and minocycline (75%). Furthermore, cross-resistance including tetracyclines (minocycline and tetracycline) and fluoroquinolones (nalidixic acid and ciprofloxacine) was observed respectively in 75 and 12.76% of *Salmonella* tested isolates (Table 3). Another cross-resistance case including four or three drugs belonging to tetracyclines and fluoroquinolones was found in *Salmonella* isolates (Table 3). No MDR case was observed in *Salmonella* tested strains.

Antibiotic residues detected in chicken litter

Analysis of chicken litter showed that among the 12 targeted molecules, 7 including ciprofloxacine (fluoroquinolones), erythromycine and spiramycine (macrolides); oxytetracycline (tetracyclines), amoxicilline

Table 2. Antibiotic resistance profile of *E. coli* strains.

Antibiotics used	Resistance % (number of strains)	Number of drugs families
AMP	7.69 (1/13)	1
TET	76.92 (10 /13)	1
MINO	76.92 (10 /13)	1
NAL	76.92 (10 /13)	1
GM	15.38 (2/13)	1
CIP	7.69 (1/13)	1
MINO/TET	76.92 (10/13)	1
NAL/CIP	7.69 (1/13)	1
NAL/MINO/ TET	76.92 (10/13)	2
NAL/MINO/ TET/AMP	7.69 (1/13)	3
NAL/MINO/ TET/GM	15.38 (2/13)	3

AMP: Ampiciline; TET: tetracycline; MINO: minocycline; NAL: nalidixic acid, GM: gentamycine; CIP: ciprofloxacine.

Table 3. Antibiotic resistance profile of *Salmonella.*

Antibiotics used	Resistance % (number of strains)	Number of drugs families
CIP	12.76 (1/8)	1
TET	87.5 (7/8)	1
MINO	75 (6/8)	1
NAL	87.5 (7/8)	1
NAL/CIP	12.76 (1/8)	1
MINO/TET	75 (6/8)	1
NAL/CIP/MINO/TET	12.76 (1/8)	2
NAL/MINO/TET	75 (6/8)	2

TET: Tetracycline; MINO: minocycline; NAL: nalidixic acid.

(betalactamines), Sulfamethoxazole and trimethoprime (sulfonamides) were detected in all analysed samples. While, tetracycline, doxycyline, ampicilline, oxacilline and penicilline G were not detected in the poultry litter analysed in this study.

In general, in all samples analyzed, ciprofloxacine had the highest rates with values ranging from 8.41 ± 2.03 mg/kg in litter from Songon to 4.56 ± 1.20 mg/kg in samples from Anyama. For areas of Bingerville and Yopougon, ciprofloxacine residues amounts were respectively 6.66 ± 1.5 and 6.61 ± 0.192 mg/kg of litter.

For erythromycine, the values ranged from 6.74± 1.20 to 5.36 ± 0.4 mg/kg of litter while for spiramycine the rates ranged from 5.01±0.61 to 4.44±0.88 mg/kg of chicken litter. The highest macrolides amounts were observed in samples from Yopougon and the lowest in litter from Bingerville.

Among antibiotics of tetracyclines classes, only oxytetracycline was detected in the analyzed litter with values of 1.57±0.5, 0.74±0.01, 0.74±0.006 and 1.01±0.01 mg/kg for samples from Yopougon, Songon, Bingerville, and Anyama respectively. Sulfonamide concentrations were 0.15±0.001, 0.05±0.002, 0.09±0.00 and 0.151±0.3 mg/kg for Trimethoprime and 1.02±0.1, 0.09±0.002, 1.18±0.3 and 1.02±0.2 mg/kg for Sulfamethoxazole for chicken litter from Yopougon, Songon, Bingerville, and Anyama respectively.

The lowest levels of antibiotic residues were observed for amoxicillin (betalactamines) with rates of 0.015±0.0003, 0.008±0.0001, 0.010±0.0001, and 0.010±0.0005 mg/kg of litter for the areas of Bingerville, Yopougon, Songon, and Anyama, respectively.

DISCUSSION

The goal in this study was to determine the contamination level of chicken litter from Abidjan poultry farms by antibiotic residues and MDR bacteria. Thus, we isolated *Escherichia coli* and *Staphyloccocus aureus* in 100% of poultry litter samples analyzed. While Salmonella spp. was isolated with the rate at 75 %. This contamination with these bacteria is not surprising, as they are naturally colonizing the intestine of poultry and can contaminate litter via feces (Shen et al., 2023; Eltai et al., 2020).

Generally, many species of bacteria are detected in

poultry litter or in its manure around the world. Among the bacteria usually detected, *E. coli, Salmonella, Staphyloccocus, Campylobacter, Clostridium, Listeria,* and *Streptococcus* occur in levels exceeding those recommended in manure considered suitable for soil amendment (Kyakuwaire et al., 2019). These are highly pathogenic bacteria with generally health devastating effects in humans and livestock and the potential to widely spread in the environment. Since poultry litter is most often used in vegetable crops, these bacteria could potentially contaminate the vegetables and could be transmitted to the consumers.

Moreover, antimicrobial susceptibility testing showed high resistance patterns for *S. aureus* to erythromycine, tetracycline, clindamycine, trimethoprime and to tetracycline, minocycline, nalidixic acid for *E. coli* and *Salmonella* strains. The findings aligned with other reports, and this resistance pattern may result from selective pressure induced by the use of these antibiotics during breeding (Ngogang et al., 2021; Mund et al., 2017). The overuse of antibiotics is considered to be the key factor promoting the emergence, selection, and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Ungemach et al., 2006). As described in other studies, *E. coli* and *S. aureus* from litter in this study were found to be highly MDR suggesting use of many drugs during breeding (Ngogang et al., 2021; Goualie et al., 2020; Rahman et al., 2020; Bodering et al., 2017). This can also be explained by the fact that these bacteria are susceptible to antibiotic selective pressure and therefore development of resistance. As *E. coli* is considered to be resistance genes reservoirs, MDR *E. coli* found in the study indicates the risk of spread of resistance genes to other bacteria and increase of AMR both in human and animal (Ngogang et al., 2021; Kaushik et al., 2018).

Additionnaly, Atidégla et al. (2016) demonstrated that application of contaminated poultry manure to the crops in gardening sites may result in contamination of vegetables by fecal bacteria including fecal coliforms, *E. coli*, and fecal streptococci. As these bacteria can persist for several months in the environment, assessment of their presence and persistence on crops and soils following use of poultry manure will be relevant as this can reveal a hidden One Health threat, especially in these cases where no decontamination is performed prior to use of litter as fertilizer. Overuse of antibiotics does not only favor the risk of emergence and transmission of MDR bacteria, but it also poses a problem of food and environmental contamination with antibiotic residues that can create severe threats for humans, animals and the environment.

The analyses carried out on samples collected showed the presence of various antibiotic residues mainly ciprofloxacine, erythromycine, spiramycine, and oxytétracycline in poultry litter generally used as manure for soil fertilization without preliminary treatment in our country. The contamination of poultry litter by these antibiotic residues is probably due to the use of these drugs during breeding. Indeed, approximately 50 to 80% of the antibiotics consumed are directly excreted by the organism. So, antibiotic residues are generally detected in soil, some waste or water at sometimes high levels (Ben et al., 2019; Madikizela et al., 2017; Díaz-Cruz et al., 2008). On the other hand, it is estimated that the use of antibiotics worldwide is between 100,000 and 200,000 tons/year, and about 50% are used in veterinary medicine. This high consumption of antibiotics leads to an increase in antibiotic residues in the environment (Madikizela et al., 2017). Since poultry litter is usually use as soil fertilizer, the presence of these molecules in high concentration observed in this study could lead to a modification of soil microbial biodiversity (Li et al., 2008). Indeed, after exposure to a mixture of 16 antibiotics (concentrations between 0.005 and 1.5 µg/L), Proia et al. (2013) observed alterations in the composition in the soil bacterial community. In addition, Laverman et al. (2015) demonstrated a change in bacterial community structure exposed to vancomycin (1000 µg/L) in river sediments downstream of wastewater treatment plants. Another consequence of the presence of antibiotics in poultry litter used as fertilizer is the development of antibiotic resistance in the natural microbial communities of these habitats (Bengtsson-Palme and Larsson, 2016). Finally, the mixing of several drugs in poultry litter observed in this study could result in significant toxicity to soil organisms or potentially interact with each other with a greater effect (synergistic effect) on the environment (Guo et al., 2017). In addition, the modification of soil microbial biodiversity, we can also supposed that these antibiotic residues could contaminate crops and then unfortunately consumers.

Conclusion

The study found high contamination of poultry litter by *S. aureus, E. coli* and *Salmonella* spp. Many strains of *S. aureus* tested were MDR against at least three classes of antibiotic. The findings also indicate that the analyzed poultry litter is contaminated with various antibiotic residues. Since no treatment is generally performed before using poultry litter as manure, it could be a source of environmental and crop contamination with MDR bacteria and antibiotic residues. Thus, the results of this study indicate that the direct use of poultry litter as a fertilizer can pose a significant risk to the environment and health for farmers, local populations, and consumers of vegetables produced in the Abidjan area.

Therefore, it is urgent, first, to impose proper antibiotic use in the poultry sector to reduce the emergence of MDR in this sector, generally and particularly in waste such as chicken litter. The results of this study also emphasize the importance of applying biological

treatments, such as composting, before using chicken litter for soil fertilization. Indeed, the use of properly composted poultry manure with selected microorganisms should help reduce the number of pathogens and the amount of antibiotic residues, thus avoiding the application of contaminated manure.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support given by FONSTI (Fund for Science, Technology and Innovation), under the grant number FONSTI/043-21/SY/OZ. They are also grateful to all farmers and field staff from the IPRAVI association who participated in this study.

REFERENCES

Atidégla SC, Huat J, Agbossou EK, Saint-Macary H, Kakai RG (2016). Vegetable Contamination by the Fecal Bacteria of Poultry Manure: Case Study of Gardening Sites in Southern Benin. International Journal of Food Science volume 2016, Article ID 4767453. 8 pages. http://dx.doi.org/10.1155/2016/4767453.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 36:493-496.

Ben Y, Fu C, Hu M, Liu L, Wong MH, Zheng C (2019). Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environmental Research 169:483-493.

Bengtsson-Palme J, Larsson DGJ (2016). Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environment International 86:140-149.

Bodering A, Ndoutamia G, Ngandolo BN, Ngakou A (2017). Antibiotic use and resistance profile of *Salmonella spp*. and *E. coli* strains isolated from poultry farms in N'Djamena and Doba cities in Chad (Article in French). International Journal of Biological Chemistry 11(4):1669-1684.

CASFM/EUCAST (2022). Comité de l'antibiogramme de la Société Française de Microbiologie. Recommandations 2022 V.1.0 Mai. 183p. Available at: https://www.sfm-microbiologie.org/wp-content/uploads/2022/05/CASFM2022_V1.0.pdf.

Dahshan H, Abd-Elall AMM, Megahed AM, Abd-El-Kader MA, Nabawy EE (2015). Veterinary antibiotic resistance, residues, and ecological risks in environmental samples obtained from poultry farms, Egypt. Environmental Monitoring and Assessment 187(2). https://doi.org/10.1007/s10661-014-4218-3.

Díaz-Cruz MS, García-Galán MJ, Barceló D (2008). Highly sensitive simultaneous determination of sulfonamide antibiotics and one metabolite in environmental waters by liquid chromatography–quadrupole linear ion trap–mass spectrometry. Journal of Chromatography A1193:50-59.

Eltai NO, Yassine HM, El-Obeid T, Al-Hadidi SH, Al Thani AA, Alali WQ (2020). Prevalence of antibiotic-resistant *E. coli* isolates from local and imported retail chicken carcasses. Journal of Food Protection 83:2200-2208.

Goualié GB, Bakayoko S, Coulibaly KJ (2020). Practices of biosecurity measures and their consequences on poultry farms in Abidjan district Food and Environment Safety 29(1):84-91.

Guo J, Li J, Chen H, Bond PL, Yuan Z (2017). Metagenomic analysis reveals wastewater treatment plants as hotspots of antibiotic resistance genes and mobile genetic elements. Water Research 123:468-478.

Inter Profession Avicole Ivoirienne (IPRAVI) (2021). La lettre avicole, the Côte d'Ivoire poultry industry newsletter (Article in French). Special issue 8:21.

Kaushik M, Kumar S, Kapoor RK, Virdi JS, Gulati P (2018). Integrons in *Enterobacteriaceae*: Diversity, distribution and epidemiology. International Journal of Antimicrobial Agents 51:167-176.

Kyakuwaire M, Olupot G, Amoding A, Nkedi-Kizza P, Basamba TA (2019). How Safe is Chicken Litter for Land Application as an Organic Fertilizer? A Review. International Journal of Environmental Research and Public Health 16:3521.

Laverman AM, Cazier T, Yan C, Roose-Amsaleg C, Petit F, Garnier J, Berthe T (2015). Exposure to vancomycin causes a shift in the microbial community structure without affecting nitrate reduction rates in river sediments. Environmental Science and Pollution Research 22:13702-13709.

Li D, Yang M, Hu J, Ren L, Zhang Y, Li K (2008). Determination and fate of oxytetracycline and related compounds in oxytetracycline. Environmental toxicology 27:80-86.

Madikizela LM, Tavengwa NT, Chimuka L (2017). Status of pharmaceuticals in African water bodies: occurrence, removal and analytical methods. Journal of Environmental Management 193:211-220.

Merchant LE, Rempel H, Forge T, Kannangara T, Bittman S, Delaquis P, Topp E, Ziebell KA, Diarra MS (2012). Characterization of antibiotic-resistant and potentially pathogenic *E. coli* from soil fertilized with litter of broiler chickens fed antimicrobial-supplemented diets. Canadian Journal of Microbiology 58:1084-1098.

Mund M, Khan UH, Tahir U, Mustafa B, Fayyaz A (2017). Antimicrobial Drug Residues in Poultry Products and Implications on Public Health: A Review. International Journal of Food Properties 20:1433-1446.

National Agency for Support to Rural Development (ANADER) (2018). Ivorian poultry days 2018: ANADER stands by the breeders (Article in French). Available at: http://www.anader.ci/journees_ivoiriennes_aviculture_2018.html.

Ngogang MP, Ernest T, Kariuki J, Mouliom Mouiche MM, Ngogang J, Wade A, van der Sande MAB (2021). Microbial Contamination of Chicken Litter Manure and Antimicrobial Resistance Threat in an Urban Area Setting in Cameroon. Antibiotics 10(1):20.

Oliveira M, Vinas I, Usall J, Anguera M, Abadias M (2012). Presence and survival of *E. coli* O157:H7 on lettuce leaves and in soil treated with contaminated compost and irrigation water. International Journal of Food Microbiology 156:133-140.

Proia L, Lupini G, Osorio V, Pérez S, Barceló D, Schwartz T, Amalfitano S, Fazi S, Romaní AM, Sabater S (2013). Response of biofilm bacterial communities to antibiotic pollutants in a Mediterranean river. Chemosphere 92:1126-1135.

Rahman MM, Husna A, Elshabrawy HA, Alam J, Runa NY, Badruzzaman ATM, Banu NA, Al Mamun M, Paul B, Das S, Rahman MDM, Mahbub-E-Elahi ATM, Khairalla AS, Ashour HM (2020). Isolation and molecular characterization of multidrug-resistant *E. coli* from chicken meat. Scientific Reports 10:21999.

Roth N, Käsbohrer A, Mayrhofer S, Zitz U, Hofacre C, Domig KJ (2018). The application of antibiotics in broiler production and the resulting antibiotic resistance in *E. coli* : A global overview. Poultry Science 98:1791-1804.

Shen X, Yin L, Zhang A, Zhao R, Yin D, Wang J, Dai Y, Hou H, Pan X, Hu X, Zhang D, Liu Y (2023). Prevalence and Characterization of *Salmonella* Isolated from Chickens in Anhui, China. Pathogens 12(3):465.

Ungemach FR, Müller-Bahrdt D, Abraham G (2006). Guidelines for prudentuse of antimicrobials and their implications on antibiotic usage in veterinary medicine. International Journal of Medical Microbiology Supplements 296(S2):33-38.

Viegas C, Carolino E, Malta-Vacas J, Sabino R, Viegas S, Veríssimo C (2012). Fungal Contamination of Poultry Litter: A Public Health Problem. Journal of Toxicology and Environmental Health - Part A75:1341-1350.

Vol. 17(12), pp. 291-305, December 2023
DOI: 10.5897/AJMR2023.9726
Article Number: D41D60A71636
ISSN: 1996-0808
Copyright©2023
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Molecular identification of biofilm-producing *Bacillus* species and yeasts isolated from food sources and their interaction with *Lysinibacillus louembei* strain

Moïse Doria Kaya-Ongoto[1,2], Christian Aimé Kayath[1,2]*, Nelly Josiane Awah-Lekaka Niebi[1] and Etienne Nguimbi[1,2]

[1]Laboratory of Cellular and Molecular Biology (BCM), Faculty of Science and Technology, Marien N'gouabi University, BP. 69, Brazzaville, Congo.
[2]National Institute for Research in Exact and Natural Sciences (IRSEN), Avenue de l'Auberge Gascogne, B.P 2400, Brazzaville, Congo.

Received 11 October, 2023; Accepted 1 December, 2023

The microorganisms contained in fermented foods are known to harbor metabolic products, possibly improving human and animal health. However, despite several studies on the functional effects of fermented foods, isolation and identification of the effective *Bacillus* species strains are still in progress. The objective of this study was to molecularly identify biofilm-producing *Bacillus* spp. (BPB) and yeasts from fermented food sources and to study their interactions with the *Lysinibacillus louembei* strain. A total of 133 isolates of *Bacillus* as well as 32 isolates of yeast were obtained for detailed identification and investigation. Based on a combination of phenotypic and molecular characterization using *fibE* polymerase chain reaction (PCR) multiplex and ITS-PCR techniques, species of *Bacillus* were identified as *Bacillus pumilus* (12%), *Bacillus subtilis* (12%), *Bacillus safensis* (6%), *Bacillus amyloliquefaciens* (6%), *Bacillus licheniformis* (6%), and *Saccharomyces cerevisiae* (0.05%). The *yfi*Q, *eps*H, *ymc*A, and *tas*A genes involved in the biofilm formation process were amplified by using PCR multiplex in *B. subtilis*, *B. licheniformis*, and *B. pumilus* have been identified and confirmed. As a phenotypic result, 45% of isolates of BPB by using the Congo Red Agar method (CRA) have been identified. The ability of *Bacillus* and yeasts to produce biosurfactants was tested by using the emulsification index (EI24). 65 and 69% of *Bacillus* and yeast isolates were able to emulsify petrol. 56% of the crude extract of biosurfactants from *Bacillus* isolates demonstrated antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* species cultures were done between *Bacillus* spp., *S. cerevisiae*, and *L. louembei*. As a result, commensalism-like interactions were obtained in yeast strain V3 and *B. pumilus* strain VB15 and *L. louembei* and *B. amyloliquefaciens*, competition-like interactions in *S. cerevisiae* strain P3 and *Bacillus* spp. strain VP11, and amensalism-like interactions with *B. pumilus* and *S. cerevisiae* and *Bacillus* spp. strain VP34 and *S. cerevisiae* strain P1. These results illustrate that microorganisms maintain different relationships that occur during fermentation process.

Keys words: *Bacillus*, *Saccharomyces cerevisiae*, *Lysinibacillus louembei*, fermented foods, microorganism interactions, biosurfactants, biofilms.

INTRODUCTION

Fermentation of various food stuffs by microorganisms is one of the oldest forms of food biopreservation (Diaz-

Munoz et al., 2022; Mgbodile and Nwagu, 2023), and leads to various products (Mgbodile and Nwagu, 2023). As previously reported, fermented foods are ecological niches abounding in significant biodiversity of microorganisms. It represents an asset for the daily meals of households. Lactic acid bacteria (LAB), *Bacillus* species, and yeasts are the most commonly found in fermented foods (Kayath et al., 2020). In the same ecosystem, *Bacillus subtillis*, *Bacillus pumilus*, *Bacillus mojavensis*, *Bacillus safensis*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis,* and *Bacillus altitudinis* could be isolated (Kimura and Yokoyama, 2019). Microorganisms including *Bacillus*, LAB, and yeasts are able to produce biosurfactant-like molecules that strongly contribute to the biopreservation of fermented foods (Elenga-Wilson et al., 2021; Marchut-Mikolajczyk et al., 2021; Stancu, 2020; Wu et al., 2022). Studies showed that *Lysinibacillus louembei* is able to produce various biomolecules, including biosurfactants, proteases, cellulases, and amylases (Kaya-Ongoto et al., 2020). Fermented foods are produced through controlled microbial growth, and the conversion of food components through enzymatic action. The microorganisms are able to produce proteases, amylases, cellulases, and pectinases that are important for a couple of biotechnological interests (Dai et al., 2020; Rajesh and Gummadi, 2022). Many studies have demonbstrated that many bacteria are also able to participate in the formation of molecular complexes such as biofilms (Arnaouteli et al., 2021; Qin et al., 2022).

Biosurfactant-like molecules could accumulate at the interface area between liquid phases and hydrogen bonding. In the food industry, biosurfactants are widely used for their properties in food preservation (Anjum et al., 2016). Microorganisms live in association by building multicellular tissues called biofilms. Biofilm-like structures involve different mechanisms and can harbor one or more species of bacteria and yeasts (Bisht et al., 2023; Zara et al., 2020). *B. subtilis* is widely used as a model organism to study the formation and characteristics of bacterial biofilms (Arnaouteli et al., 2021; Qin et al., 2022). Some genes have been found to be involved in the biosynthesis of biofilm molecules. Loci involved in biofilm formation are generally named *eps* or *cps* in *Bacillus*. Other loci for polysaccharide production could be localised on plasmids in *Lactococcus* and *Bacillus* spp. In *B. subtilis,* exopolysaccharide matrix is under the expression of the *eps*A-O locus (Irnov and Winkler, 2010). One of the genes of this locus, *eps*E*,* encodes a bifunctional protein, which is at the same time a glycosyltransferase involved in the inhibition of mobility by blocking flagellar rotation. Four genes (*eps* H, I, J, and K) of this locus intervene in the production of N-acetyl-galactosamine (Blair et al., 2008).

Microbial interactions have been described many years ago (Schroeckh et al., 2009). Some of them have been qualified as direct and indirect interactions (commensalism, mutualism, competition, amensalism, parasitism, etc.) resulting in the production of compounds (Sieuwerts et al., 2008). During fermentation, microorganisms coexist and interact with each other to lead to a quality end product (Kayath et al., 2020; Kimura and Yokoyama, 2019).

Yeasts have been shown to be involved in increasing polyphenols compounds and may also interact synergistically with *Bacillus* spp. (Kayath et al., 2020). To our knowledge, interaction between bacteria and yeasts remains a big challenge. Many previous studies did not clearly explain the molecular interactions in fermented foods. The objective of this study was to molecularly identify biofilm-producing *Bacillus* spp. (BPB) and yeasts isolated from food sources and to study their interaction with the *L. louembei* strain.

METHODS

The microorganisms tested in this study were collected from fermented foods and beverages, including palm wine (*Nsamba (VP)*), fermented cassava leaves (*Ntoba mbodi (NM)*), banana wine (*Mbamvu (VB)*), and ginger wine (G) (*Tangawiss* as local name). *L. louembei* has been previously isolated in our laboratory (Ouoba et al., 2015).

Isolation and characterization of *Bacillus* and yeasts strains

Ten-fold serial dilutions of each sample including VP, NM, VB, and G were prepared in sterilized distilled water. For *Bacillus* isolation, isolation and characterization have been previously described (Elenga-Wilson et al., 2021). For yeast isolation, decimal dilutions were inoculated on Sabouraud agar medium supplemented with 0.1 mg/L of chloramphenicol. Incubation was done at 30°C for 24 to 48 h. Each colony associated with different phenotypic characteristics was separately isolated. The purification of isolates was carried out as in the case of Bacilli. Purity was estimated by using microscopic observations in terms of morphological characterizations.

Detection of *Bacillus* spp. ability of biofilms formation

Phenotypic characterization of slime-producing ability

Detection of biofilm formation using Congo Red Agar has been evaluated according to a modified and adapted protocol recommended as previously discribed (Freeman et al., 1989). This method consists of the cultivation of isolates on Congo Red Agar. The Congo Red Agar (CRA) medium is composed of 23.5 g/L PCA medium, 50 g/L sucrose, and 0.8 g/L Congo Red dye. Congo Red has been separately prepared from other constituents of the medium in the form of a concentrated aqueous solution and then autoclaved; then it was added when the agar cooled to around

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

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Table 1. Primers used for PCR amplification of encoding internal spacers transcribed in yeasts.

Primers	Sequence
ITS1	5'-TCCGTAGGTGAACCTGCGG-3'
ITS4	5'- TCCTCCGCTTATTGATATGC-3'

55°C. The different isolates were inoculated by streak seeding. Petri dishes were incubated at 37°C for 24 h. The ability to form biofilm is revealed by the presence of black colonies on a lens of dry consistency.

Production of biofilms by using crystal violet

A qualitative method for evaluation of biofilm formation has been used (Christensen et al., 1982). Each *Bacillus* isolate was cultured in 10 mL of Trypticase Soy Broth (TSB) supplemented with 2% glucose (TTM), the whole being contained in tubes. For 24 to 48 h, the tubes are incubated aerobically at 37°C. The tubes were then gently decanted by tapping them and washed with PBS pH 7.3, and stained with 2% crystal violet (gentian violet). After incubation at 37°C for 30 min, tubes were rinsed with distilled water and dried in the reverse position on the bench. The biofilm formation is signed as positive when a visible film lines the wall and/or the bottom of the tubes, which gives a blue-purplish coloration. Tubes were examined and biofilm formation was noted as absent (-), weak or moderate (+), and strong (++). The test was performed in triplicate for each isolate.

Ability of *Bacillus* and yeasts to produce biosurfactants

Ability of *Bacillus* isolates and yeasts to produce biosurfactants have been done as previously experimented (Elenga-Wilson et al., 2021). E24 represents the emulsification index which is the total height of the hydrocarbon (Ht) and the height of the emulsion (He) was measured using a graduated ruler. The values obtained allowed the calculation of the emulsification index E_{24} according to the formula:

$$E_{24} = \frac{HE}{HT} \times 100$$

where He= height of emulsion layer; Ht= total height of solution; E_{24}= emulsion index for 24 h.

Biosurfactant extraction by chloroform and ammonium sulfate

First of all, 5 mL of overnight culture were fuged. The supernatant coming from each isolate was added to an equal volume of chloroform (v/v). The mixture is strongly agitated by a vortex. After centrifugation at 6.000 rpm for 10 min, the non-aqueous phase is recovered. Evaporation of the solvent was completely done at room temperature. The residue is dissolved in a phosphate buffered saline (PBS) containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4, pH 7.0. In terms of ammonium sulfate, an overnight culture has been fudged at 13.000 rpm for 15 min to separate the supernatant and pellet. The supernatant was then mixed with ammonium sulfate (80%) for 15 min. And finally, this has been incubated overnight. The mixture has been fuged at6.000 rpm for 30 min. Pellets have been homogenized by using PBS. For both extractions (chloroform and ammonium sulfate), the emulsifying activity was tested in comparison with the supernatant at the start.

Antimicrobial activity of biosurfactant extracts

An overnight culture at 37°C of *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella* species were done on specific media including Chapman medium, EMB medium, and S.S medium as well. A fraction of each pathogen strain was scraped and mixed with physiological water until reaching an OD corresponding to 0.5 McFarland. The next step consists of inoculating with a swab the inoculum of the pathogen previously obtained in physiological water on MH medium (or PCA) by making tight streaks in three directions. The Petri dishes are then dried for 20 to 30 min at 37°C. 20 µL of biosurfactant extracts from each *Bacillus* spp. are deposited on medium where the pathogenic strain to be tested has been previously inoculated. The dishes were incubated at 37°C for 24 h, and then the diameters of the inhibition halos were measured to judge the effectiveness of the biosurfactant.

Molecular identification of *Bacillus* isolates

To target strains such as *B. amyloliquefaciens*, *B. subtilis*, *B. pumilus*, *B. licheniformis*, and *B. safensis*, a PCR multiplex using the *fibE* gene encoding for fibrinolytic enzyme was used. The *fib*E primers were taken from the work first carried out and previously discribed (Kaya-Ongoto et al., 2020) Extraction and purification of genomic DNA of isolates were performed according to the NucleoSpin Microbial DNA (Macherey-NAGEL) kit. DNA purity was assessed by electrophoresis on a 1% agarose gel and by the ratio of optical densities of 260/280 nm. The genomic DNA obtained was used as a template for all PCR amplification experiments. In terms of yeast, a modified and adapted protocol was performed in our laboratory according to the protocol described in the previous study (Dymond, 2013).

Yeast identification by using ITS PCR amplification

A PCR reaction was carried out in a final volume of 50 µL containing 17 µL of distilled water, 4 µL of DNA, 2 µL of each primer (Table 1), and 25 µL of master mix. The 0.2 mL microtubes are then placed in a thermal cycler (Biorad, Singapore). PCR conditions included initial denaturation at 95°C for 5 min, followed by 25 cycles each comprising denaturation at 95°C for 30 s, hybridization at 60°C for 30 s, an extension at 72°C for 30 s and a final extension at 72°C for 7 min.

Colony multiplex PCR amplification of *epsH*, *tasA*, and other genes in *Bacillus* spp.

Genes involved in biofilm formation were also amplified in this work using the multiplex PCR colony approach. To achieve this, cultures of each bacterium were heated at 95°C for 15 min in a volume of 15 µL of sterile distilled water with a thermocycler to break cells for releasing genetic material that will be used as a matrix. Specific primers were generated from sequences uploaded to the NCBI portal (National Center for Biotechnology Information, https://blast.ncbi.nlm.nih.gov/Blast.cgi) genomic database of targeted strains. Microbesonline (http://www.microbesonline.org) has been used for checking lists. pDRAW32 software has been used for bioinformatics analysis. These different primers are listed in Table 2. A multiplex PCR reaction was carried out in a final volume of 50 µL comprising 25 µL of master mix, 2 µL of each

Table 2. Primers used for amplification of genes involved in *Bacillus* spp. biofilms.

Primers	Sequence (5'----3')	Size (pb)	Strain
BPymcAF	ACGCTTTATTCAAAAAAAGAGATTG	435	*Bacillus pumilus*
BPymcAR	TCATTTAATGGAGCAACTTGGT		
BLymcAF	GTGACGCTTTATACGAAAAAAGAGATT	432	*Bacillus licheniformis*
BLymcAR	TTATAGAGAACAGCTCGGTGAT		
BLyfiQF	TTTTGCTTCATGCGATATCAATGG	1089	
BLyfiQR	TTAACCTGCATGATGGCGC		
BStasA1F	ATGGGTATGAAAAAGAAATTGAGTT	786	*Bacillus subtilis*
BStasA2R	TTAATTTTTATCCTCGCTATGCG		
BSepsHF	AAACACCTGCGGTTAGTCTG	1035	
BSepsHR	TCACCCTCTGTTTCTCATTTTGTA		

Table 3. Distribution of isolates obtained by sample.

Sample	*Bacillus*	Yeast
Palm wine (VP)	46	9
Ginger wine (G)	32	14
Banana wine (VB)	17	9
Ntoba mbodi (NM)	39	-
Total	134	32

primer, 16 µL of ultrapure water, and 5 µL of matrix DNA. PCR conditions included initial denaturation at 95°C for 5 min, followed by 30 cycles comprising denaturation at 95°C for 30 s, a gradient hybridization from 55 to 60°C for 30 s, an extension at 72°C for 60 s and a final extension at 72°C for 7 min. 5 µL of each amplification product was mixed with 2 µL of loading buffer (BIOKE). Mixtures were subjected to electrophoresis on 1.5% agarose gel (w/v).

Interaction between *Bacillus* spp. and other microorganisms

Coculture between Bacillus spp. and yeasts

Each isolate's colony fraction was scraped off the agar and launched separately into 50 mL of nutrient broth, where it was incubated at 37°C at 150 rpm. To determine and/or to compare the type of interaction, 0.5 mL of each exponential growth culture was innoculated in single and mixed cultures. During growth, the enumeration in CFU.mL^{-1} of microorganisms has been done every 12 h. For yeast enumeration, Sabouraud agar medium supplemented with 0.1 mg/L of chloramphenicol has been used, and Mossel agar medium for *Bacillus* spp. Each experiment is done in triplicate.

Coculture between B. amyloliquefaciens-NM11 and L. louembei

The same protocole has been used. The Mossel medium was only used to enumerate the flora of *B. amyloliquefaciens* and *L. louembei* after incubation aerobically at 37°C. For *L. louembei*, CFU was then determined by subtracting the total CFU of coculture with *B. amyloliquefaciens* and CFU was found in coculture on Mossel medium supplemented with 6 mg/mL of gentamicin (an antibiotic used for the selection *B. amyloliquefaciens*). Each experiment was done in triplicate.

RESULTS

Isolation and characterization of *Bacillus* and yeasts strains

From the biological materials used, a total of 134 isolates were obtained from Mossel medium, of which 46 were obtained from palm wine samples, 17 from banana wine, 32 from ginger juice, and 39 from fermented cassava leaves. However, on Sabouraud + chloramphenicol medium, a total of 32 isolates were obtained, including: 9 from palm wine samples, 9 from banana wine, and 14 from ginger juice. As for the 32 yeast isolates, the characterization indicates that all of these yeasts divide by budding and are immobile. The majority (60%) of the cells were ovoid in shape (Table 3) with whitish coloration, regular outline and creamy consistency.

Detection of *Bacillus* spp. capacity to form biofilms

Phenotypic characterization of slime-producing ability

The test of biofilm production by the Congo Red Agar

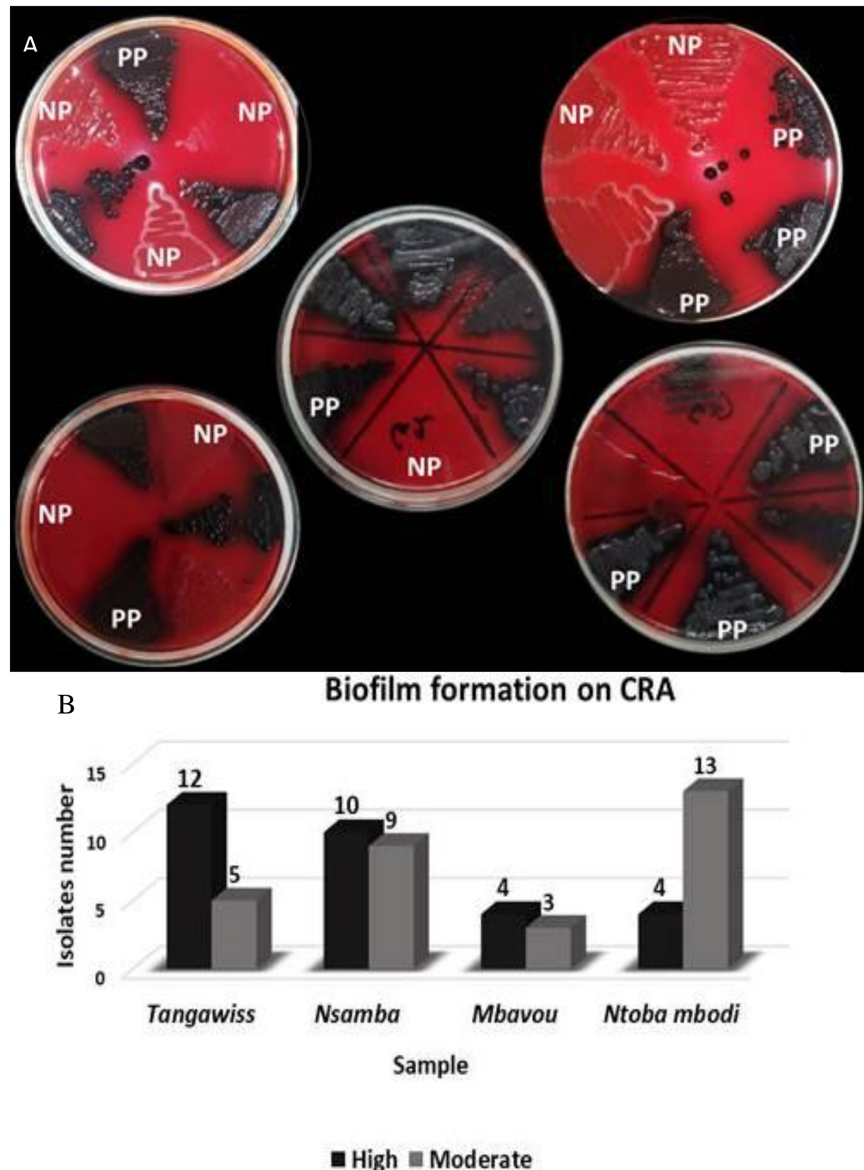


Figure 1. A: Capacity of strains for biofilm formation on Congo Red Agar (CRA) by *Bacillus* spp. NP: Negative phenotype, PP: positive phenotype. B: biofilm partitioning based on sample and phenotype.

method showed that, out of a total of 133 isolates tested, we have 60 isolates or 45% were positive. This positive phenotype is thus reflected by black colonies (strong or moderate coloration) with a crystalline lens of dry consistency on Congo Red Agar after incubation for 24 h at 37°C. This aspect is due to the reaction between Congo Red and exopolysaccharides produced by the isolates tested. 73 isolates or 55% were nonproducers of biofilms, presenting red colonies on the same agar medium. The phenotypic aspect of these results is as shown in Figure 1A. Additionally, Figure 1B shows the repartition of isolates capable of producing biofilm per sample.

Methods for producing biofilms in a tube and staining with crystal violet/gentian violet

Twenty one positive isolates with a better profile of biofilm formation on Congo Red Agar (CRA), as well as 25 isolates negative for the CRA test, were selected for this test. Among the 21 isolates (previously positive for CRA) tested, 81% or 19 isolates remained positive (15++ and 2+), while 19% or 2 isolates were negative. On the other hand, of the 25 isolates negative for CRA, 36% or 9 isolates were positive (+) while 64% or 16 isolates remained negative. However, 9 isolates which were found to be positive when performing the test tube

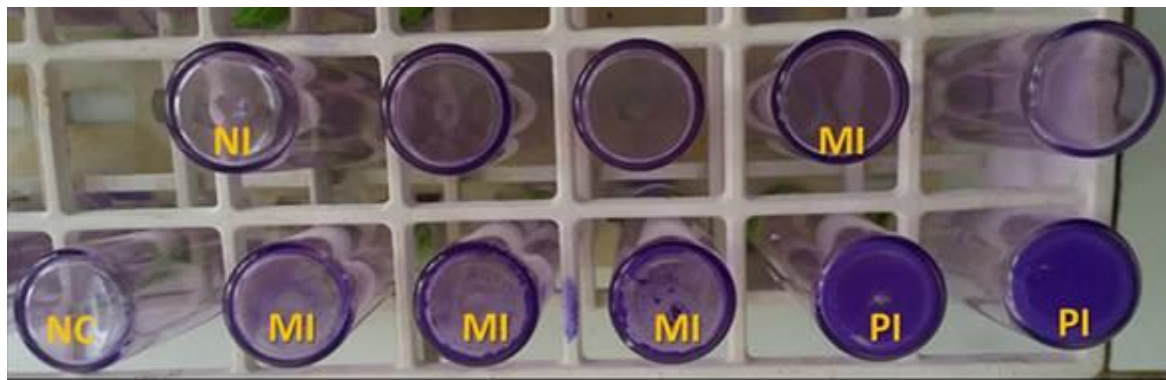


Figure 2. Screening of biofilm production by the tube method (TTM). Scores of 50 and 100%, were respectively, assigned to the MI: moderate isolate and PI: positive isolate. 0 was assigned to the isolate with negative results (Table 4). NI: Negative control, NC: negative control.

Table 4. Biofilm formation using TTM.

***Bacillus* isolates**	**Result**	**Assigned score (%)**
G1, G5, VB11, VB18, NM14, NM53, VP16	Positive and strong	100
VP2, VP21, G14, G18, VB10, NM22	Positive and moderate	50
G6, G13, G20, G21, VP18, VP19	Negative	0

method with Trypticase Soy Broth supplemented with glucose were again negative for the test tube method using TSB supplemented with sucrose. Biofilm formation resulted in a film lining the bottom of the test tube and/or the wall (Figure 2 and Table 4).

Testing of *Bacillus'* ability and yeasts to produce biosurfactants

Isolates of *Bacillus* spp. have also shown a good ability to produce biosurfactants. At the end of the two methods used, we thus determined the emulsification index after 24 h (EI24), in particular, first from total culture, then from the cell-free supernatant after centrifugation. Consequently, out of the 60 isolates tested (able to form biofilm), 39 isolates or 65% were able to emulsify the gasoline with an EI24 between 5 and 100% after 24 h of incubation at room temperature. 11 of the 39 positive isolates obtained an EI24 ≥ 50% with the total culture, and 5 other isolates obtained an emulsification index EI24 ≥ 50% with the cell-free supernatant. This ability results in an emulsion of the fuel used (Figure 3A). The various indices obtained from the strains tested are represented in Figure 3B.

Antimicrobial activity of biosurfactant extracts

Biosurfactants have been reported to have antimicrobial effects. In this study, *Bacillus* isolates showed good efficacy against *E. coli*, *Salmonella* spp., and *S. aureus* when taken as models of pathogens. Among the 16 isolates tested, 9 or 56% showed an inhibitory effect against the various pathogenic bacteria used in this study. Indeed, 6 extracts of biosurfactants were able to inhibit the growth of *S. aureus* and *Salmonella* spp., and 5 extracts were able to inhibit *E. coli* (Figure 4).

Molecular identification of *Bacillus* and yeast isolates

To confirm the identity of the 16 isolates presenting both better profiles in terms of their ability to produce biofilms and to emulsify hydrocarbons with an EI24 ≥ 50%, amplification of the *fib*E gene was carried out. A size of about 850 bp was obtained for the primers used, like *fib*EBs (targeting *B. subtilis*), *fib*EBp (targeting *B. pumilus*), *fib*EBsa (targeting *B. safensis*) and *fib*EB1 (targeting *B. licheniformis*). Isolates were identified as *B. pumilus* (12%), *B. subtilis* (12%), *B. safensis* (6%), *B. amyloliquefaciens* (6%), and *B. licheniformis* (6%) (Figure 5A). PCR performed allowed amplification of the universal genes encoding internal spacers transcribed in yeast isolates. In total, 8 strains were able to amplify the targeted gene. The amplicons revealed unique bands around the expected sizes. For all isolates selected, six including S1, S2, and S3 were able to amplify ITS 1/4 gene, with a band around 650 bp; on the other hand, P1 and P3 from palm wine were able to amplify the same

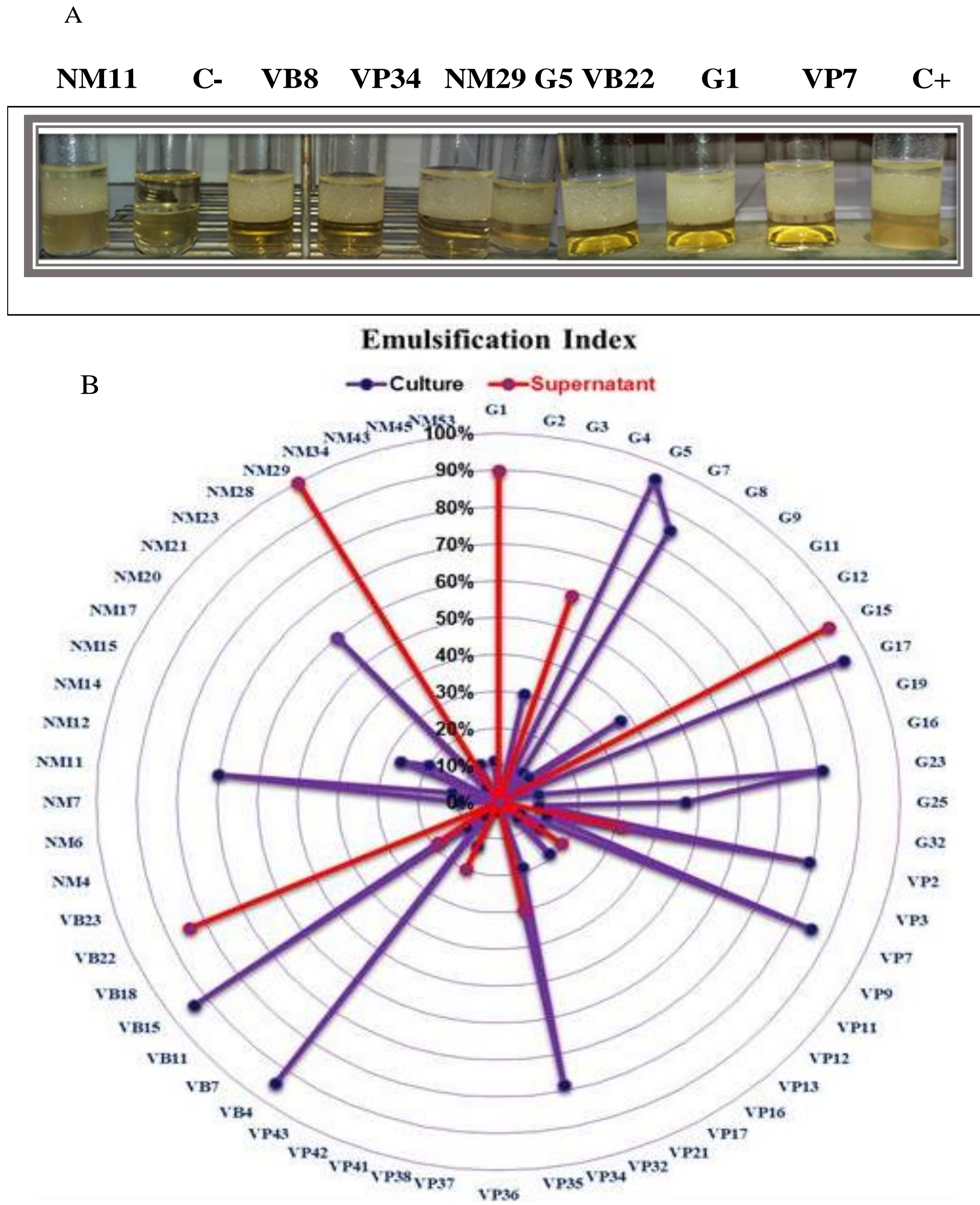


Figure 3. A: Emulsion of gasoline by some isolates of *Bacillus* spp. B: Emulsion index –(EI24) of *Bacillus* spp. Isolates C-: negative control; C+: positive control, *Bacillus* isolates NM4, 6, 7, 11, 12, 14 15, 17, 20, 21, 23, 28, 29, 34, 45, 53. G1, 2, 3, 4, 5, 7, 8, 9, 11, 12, 15, 16, 17, 19, 23, 25, 32. VB4, 7, 11, 15, 18, 22, 23, and VP2, 3, 7, 9, 11, 12, 13, 16, 17, 21, 32, 34, 35, 36, 37, 38, 41, 42, 43.

gene with a band around 850 bp. Figure 5B illustrates the electropherogram resulting from these amplifications.

Multiplex colony PCR of genes involved in biofilms

To demonstrate the presence of the genes encoding biofilm markers, multiplex PCR targeting *eps*H, *tas*A, *ymc*A, and *yfi*Q genes has been performed. *Bacillus* isolates VB15 and G23 were identified as *B. pumilus; Bacillus* isolates G7 and G26 were identified as *B. subtilis;* and *Bacillus* isolate G33 was identified as *B. licheniformis eps*H and *tas*A genes were positive in *B. subtilis*. *ymc*A and *yfiQ* genes were positive in *B.*

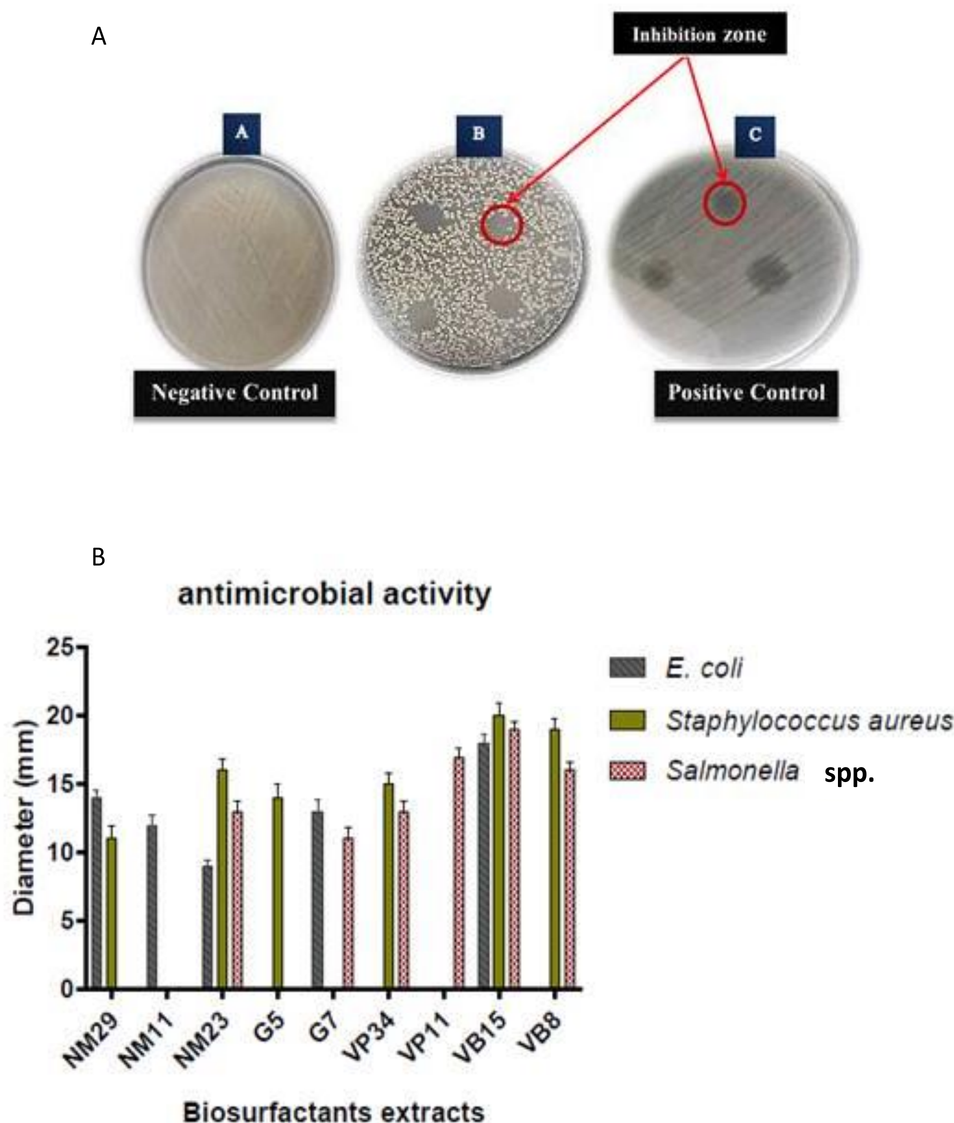


Figure 4. Antimicrobial activity of *Bacillus* biosurfactants on pathogenic models. A: activity illustration, B: Diameters of inhibition. MM29, MN11; MN23: Isolates from Ntoba Mbodi sample. G5, G7: Isolates from ginger wine. VP34, VP1: Isolates from palm wine. VB15 and VB8: Isolates from Banana wine.

licheniformis. *ymc*A gene was positive in *B. pumilus*. Thus, the targeted genes were amplified and obtained at good sizes, as shown in Figure 6 and confirmed with controls.

Interactions between yeasts and *Bacillus* spp.

Isolates with a percentage of EI24 ranging from 50 to 100% were selected for the characterization of the type of interaction between yeasts and *Bacillus* spp. The difference between the growth in single culture (pure culture) and coculture was evaluated after enumeration by comparing the values of the growth rates during time.

Coculture between Bacillus spp. strain VP11 and S. cerevisiae strain P3

Both strains were isolated from palm wine. The values of the growth rate in CFU/mL were multiplied by the "logarithm". Thus, the analysis of the growth curves in single and in coculture (Figure 7A) shows that in coculture as in single culture, the two isolates follow the same growth logic, with a slight regression in the growth rate (Log CFU/mL) during time in coculture compared to

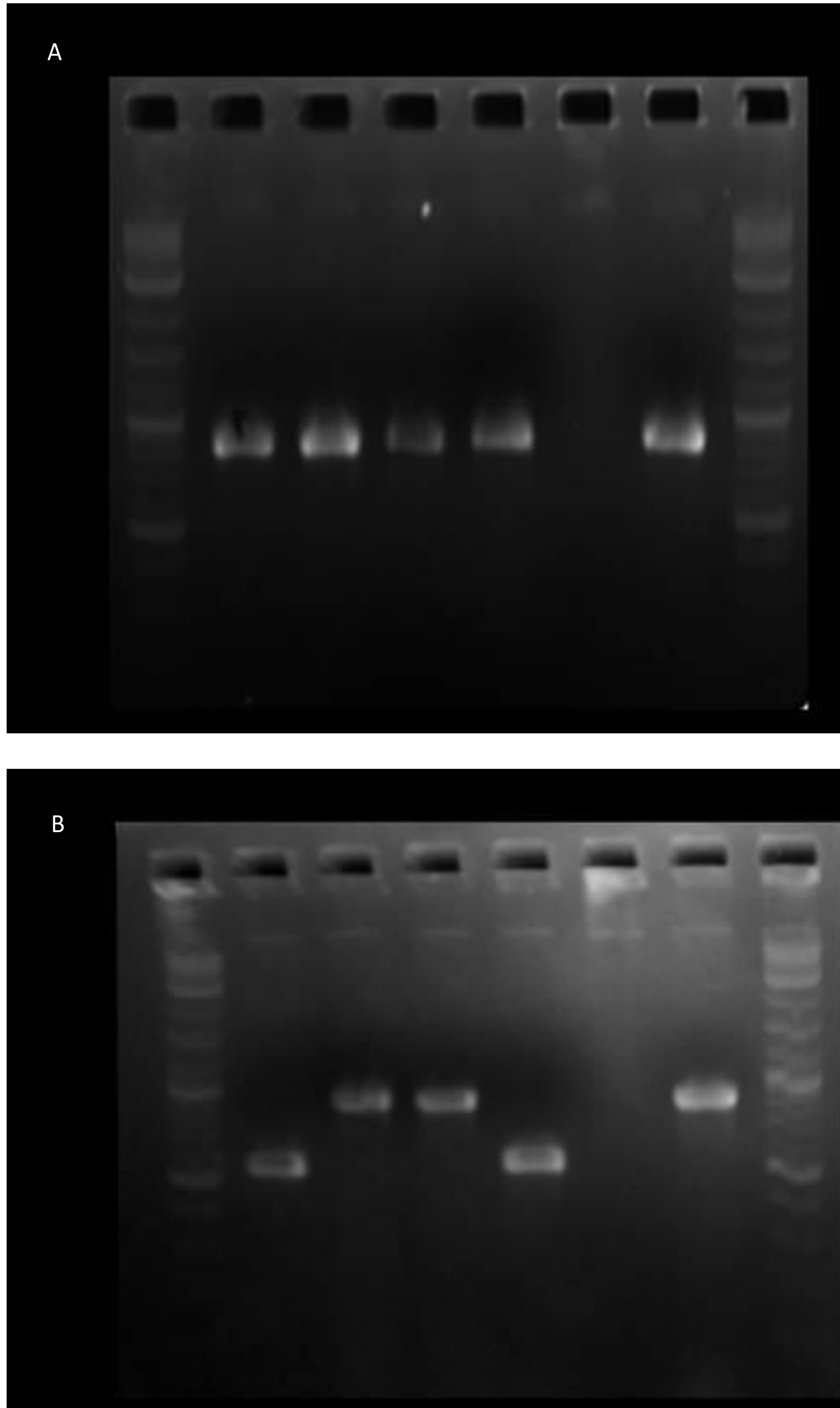


Figure 5. (A): 1% agarose gel electrophoretic profile of the fibE gene PCR amplicons from *Bacillus* spp. isolates (MP: Molecular Weight Marker, lane 1: *Bacillus* spp. isolate G7, lane 2: *Bacillus* spp. isolate VB15, lane 3: *Bacillus* spp. isolate G17, lane 4: *Bacillus* spp. isolate NM23, lane 5: negative control, 6: positive control. B: Electrophoretic profile on a 1.5% agarose gel of PCR products of the ITS region of rDNA from yeast isolates. MP: molecular weight marker; lanes 1 and 2: *Bacillus* spp. isolate V1, lane 3: *S. cerevisiae* strain P3, lane 4: *S. cerevisiae* strain S2, lane 5: negative control, and lane 6: positive control.

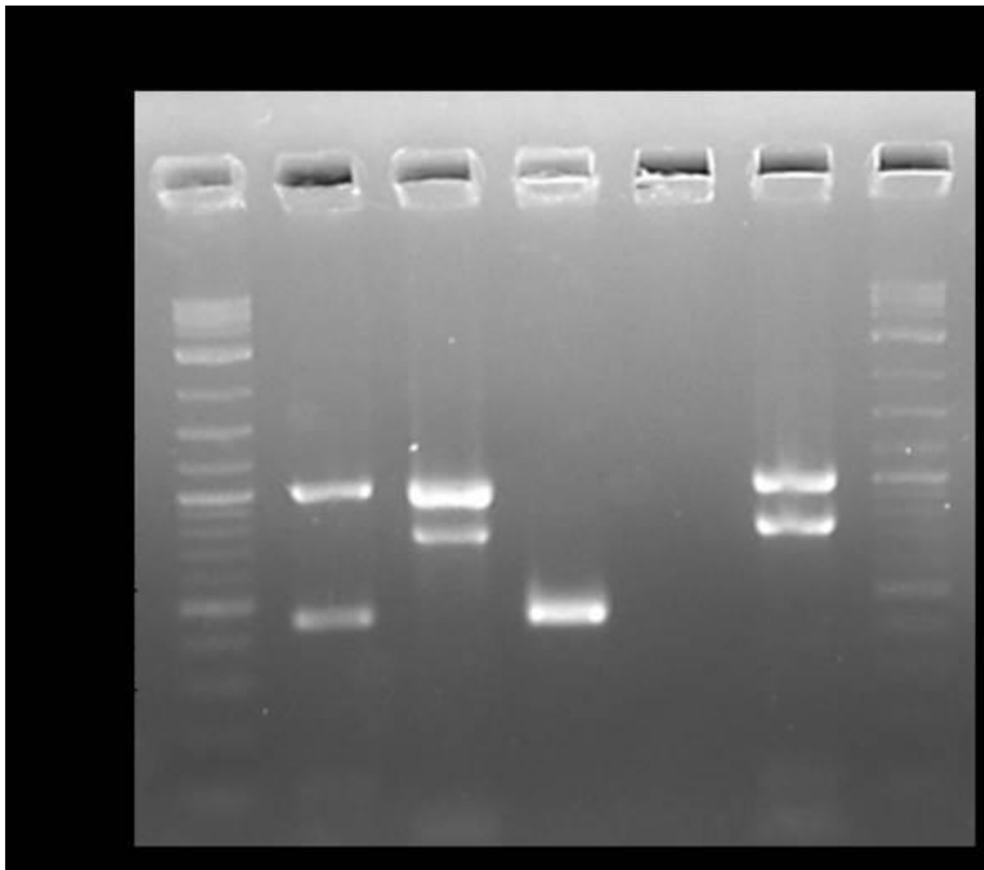


Figure 6. 2% agarose gel electrophoretic profile of PCR products of *eps*H, *tas*A, *ymc*A and *yfi*Q genes from *Bacillus* isolates. (MP: 2-Log molecular weight marker, lane 1: *Bacillus* isolates G33 (*yfi*Q≈1089 bp and *ymc*A≈432 bp genes), lane 2: *Bacillus* isolates G7 (*eps*H ≈1035 bp and *tas*A≈786 bp genes), lane 3: *Bacillus* isolates VB15 (gene *ymc*A≈435 pb), lane 4: negative control, lane 5: *Bacillus subtilis* was used as a positive control.

that observed in the single culture. This observation shows that there is an interaction between the two microbial populations (Figure 7A).

Coculture between B. subtilis strain G7 and yeast strain S2

The strains tested were isolated from *Tangawiss* (ginger juice). The second mixed culture was carried out between *B. subtilis* strain G7 and yeast isolate S2 in LB broth. The count was made with a volume of the inoculum of 100 µL. After enumeration, the analysis of the growth profiles for the pure and mixed cultures (Figure 7B) was carried out according to the same procedures as the first. Furthermore, it showed that the flora of *B. subtilis* strain G7 in coculture increased considerably compared to that in the single cultures. In addition, the yeast isolate S2 regresses slightly more in mixed culture than in single culture. It emerges from this observation that the cohabitation of the two isolates promotes the growth of G7 and that there is an interaction between the two microbial populations (Figure 7B).

Coculture between B. pumilus strain VB15 and yeast strain V3

A third mixed culture was carried out between strains V3 and VB15 from "*Mbamvu*", the growth of the two types of microorganisms was followed according to the same methods as the others. The comparison of the growth profiles showed on one hand that when V3 grows in the presence of VB15, the growth rate (Log CFU/mL) increases considerably compared to V3 in single culture and a slight decrease in the V3 flora is observed in coculture with 96 h. On the other hand, the growth of VB15 remains almost unchanged over time. This observation suggests that the growth of V3 in mixed culture is stimulated by VB15 and that there is a positive interaction between the two microbial populations (Figure 7C).

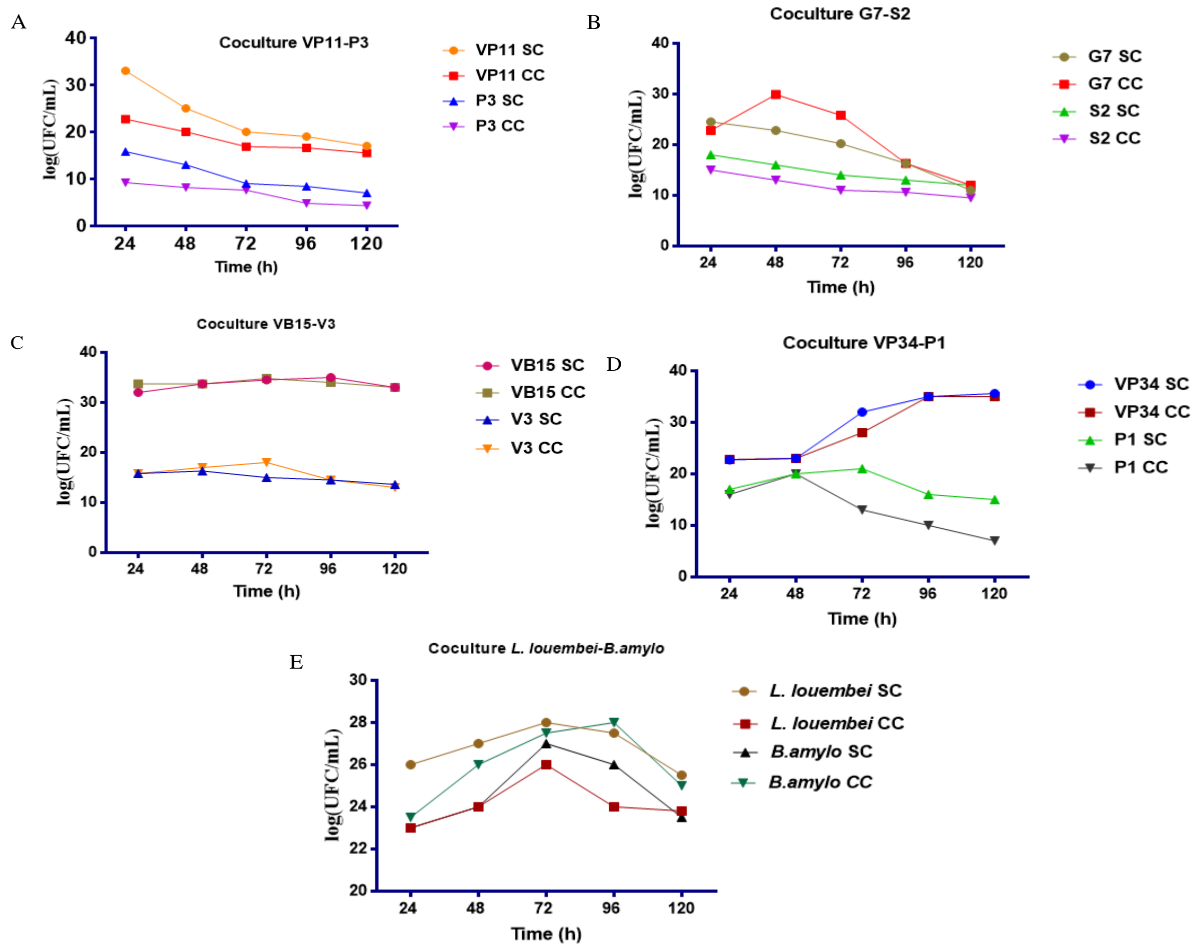


Figure 7. Single culture and coculture growth profiles. A: between *Bacillus* spp. isolate VP11 and *S. cerevisiae* isolate P3. B: between *Bacillus subtilis* strain G7 and yeast isolate S2. C: between *Bacillus pumilus* isolate VB15 and yeast isolate V3. D: between isolates *Bacillus* spp. Isolate VP34 and *S. cerevisiae* strain P1. E: *Bacillus amyloliquefaciens* strain NM11 and *L. louembei*. CC: coculture, SC: single culture.

Coculture between Bacillus spp. strain VP34 and S. cerevisiae strain P1

Two strains used were isolated from the same sample, "palm wine". In this case, the comparison of the growth profiles shows that from 48 h when P1 is in coculture with VP34, its flora regresses compared to the single culture of P1 and that the growth of VP34 in pure and mixed culture remains almost unchanged with the growing flora. This observation shows that there is an interaction between the two isolates and that the presence of VP34 in the same medium disadvantages the growth of P1 (Figure 7D*).*

Coculture between *B. amyloliquefaciens and L. louembei*

L. louembei has been previously identified and isolated from "*Ntoba Mbodi*" in our laboratory. This strain was tested in mixed culture with *B. amyloliquefaciens* (NM11) from the same sample, to study the interaction between both microorganisms. This fact bears resemblance to that observed in the case of the mixed culture of *B. pumilus* strain VB15 and yeast strain V3 *B. pumilus* strain VB15 and the yeast strain V3. This is because *L. louembei* stimulates the growth of *B. amyloliquefaciens*. This is a positive interaction during which the cohabitation of microorganisms is favourable to one of them (*B. amyloliquefaciens*) by impacting the growth of *L. louembei* (Figure 7E).

DISCUSSION

This work aimed to identify bacteria at the molecular level and to study the interactions between microorganisms isolated from 4 fermented foods found in the Republic of

Congo. 133 bacterial isolates belonging to the *Bacillus* genus and 32 isolates of yeast were obtained from fermented cassava leaves, ginger juice, palm wine, or banana wine. Several studies have shown that bacteria of the *Bacillus* genus and yeasts constitute ecological niches in fermented foods and actively participate in the fermentation quality (Ouoba et al., 2008). Fermented foods are complex ecosystems harbouring microorganisms that are able to interact with each other by producing biomolecules of interest and by forming biofilms (Marchut-Mikolajczyk et al., 2021; Qin et al., 2022; Jumpathong et al., 2022).

Biofilms are bacteria lifestyles in natural environments, and they severely impact much of the bacterial composition. In *Bacillus* spp., the extracellular matrix mainly comprises many components: exopolysaccharides (EPS), proteins (TasA, TapA, and BslA) and some nucleic acids (Pandit et al., 2020). This work showed that 45% of *Bacillus* isolates were positive on CRA. It has been shown that 60% of food-borne bacteria, including the genus *Bacillus* are capable of producing biofilm-like structures using the CRA method. In addition, some phenotypic differences have been seen, namely, the strong or moderate black coloration (Arnaouteli et al., 2021; Qin et al., 2022; Blair et al., 2008). This could be explained by the level of gene expression involved in the exopolysaccharide biosynthesis and the substrate specificity as well. This could justify the two stages of biofilm formation: adhesion to the surface followed by the production of exopolysaccharides (Pandit et al., 2020). Exopolysaccharides are multifunctional compounds that have interesting applications in both the pharmaceutical and food industries (Elzeini et al., 2021).

Sensitivity of CRA could be explained by the fact that the formation of biofilm or the production of exopolysaccharides can be conditioned by the type of substrate metabolized, especially since in this test sucrose was substituted by glucose. These results indicate that the capacity for biofilm formation depends on substrates linked to environmental factors (Xu et al., 2010).

Bacillus has long been known for its ability to produce biosurfactants such as lipopeptide, surfactin, iturin, fengycin, and lichenysin (Eras-Munoz et al., 2022). However, it has been reported that surfactin is also a quorum sensing molecule which has a positive effect on biofilm formation, especially in *B. subtilis* (Pandit et al., 2020). In this work, we demonstrate the correlation between biofilm formation and biosurfactant production. 65% of *Bacillus* isolates were able to emulsify the gasoline with an EI24 between 5 and 100% after 24 h of incubation at room temperature. Those bacteria were also capable of producing BLS. This emulsification has also been observed in bacteria such as *Pseudomonas aeruginosa, B. subtilis,* and *B. licheniformis,* which have been widely used in the bioremediation of polluted soils (Stancu, 2020; Wu et al., 2022). As for the yeasts, 69% of the isolates were able to emulsify gasoline, with an emulsifying activity of the strains ranging from 46 to 100%. Many studies have demonstrated the same phenotype (Zara et al., 2020; Jezierska et al., 2018; Konishi et al., 2011).

Isolates with an EI24 ≥ 50% were selected for biosurfactant extraction. This made it possible to confirm that these biosurfactants are extractable, as shown in the work carried out with *B. subtilis* (Cheng et al., 2013; Mnif et al., 2013). Subsequently, we showed that the extracted biosurfactants of 56% *Bacillus* isolates have antimicrobial activity, as they are able to inhibit the growth of pathogenic bacteria such as *E. coli*, *S. aureus*, and *Salmonella* spp. This is an interesting observation because *Bacillus* spp. and yeasts would play an important role in the preservation of local fermented products through their biosurfactant-like molecules, due to their antimicrobial properties. It was previously shown that the lipopeptides of *B. subtilis* exhibited antibacterial and anti-biofilm activity against opportunistic bacteria or pathogenic agents such as: *Alcaligenes faecalis*, *Achromobacter xylosoxidans*, *Pseudomonas alcaligenes,* and *Pseudomonas putida*; with partial inhibition also observed against *Klebsiella aerogenes*, *E. coli* and *Pseudomonas aeruginosa* (de Souza et al., 2020). As previously reported, *Bacillus methylotrophicus* through its BLM has antibacterial activity by protecting against *Salmonella enterica* and *Xanthomonas campestris* contamination (Rani et al., 2020).

It has recently been shown that the *fib*E gene can be used in the molecular identification of *Bacillus* spp. belonging to phylogenetic group I (Kaya-Ongoto et al., 2019). This method is reliable, fast and has great discriminatory power. On the basis of a combination of phenotypic and molecular characterisation using fibE PCR multiplex, species of *Bacillus* isolated from fermented food were identified as *B. pumilus* (12%), and *B. subtilis* (12%), *B. safensis* (6%), *B. amyloliquefaciens* (6%), and *B. licheniformis* (6%). Similar results were obtained showing the presence of *Bacillus* genera (Parkouda et al., 2009).

A new method of multiplex PCR using biofilm genes, including *eps*H, *tas*A, *ymc*A, and *yfi*Q has been found in this work. *B. subtilis, B. licheniformis,* and *B. pumilus* have been identified and confirmed. The method is correlated with the technology using *fib*E gene amplification.

To identify yeast isolates at the molecular level, the direct PCR approach for the identification of yeast species has been chosen among the techniques described in the literature for its discriminatory power and ease of implementation. In the context of this work, it was shown that *S. cerevisiae* strain P1 and strain P3 have been identified. ITS1/ITS4 of 5.8S rRNA gene with a fragment size of around 850 bp, and isolates V1, V2, and V3 from banana wine, as well as isolates S10 and S13 from ginger juice have been linked to other genera or

species of yeast. The identification of *S. cerevisiae* species showed a fragment size about 850 bp and those of other species around 650 bp (David et al., 2014). The results of the present study showed identification of *S. cerevisiae* in palm wine. Similar result has been previously obtained. The predominance of this species in palm wine is due to the high sugar and alcohol content in palm wine, unlike ginger juice and "*Mbamvu*". Unlike other species, *S. cerevisiae* can tolerate moderately high levels of sugar and alcohol (Guo et al., 2020).

Fermented foods are the products of complex interactions between molds, yeasts, and bacteria. It is therefore important to understand interactions between different groups. The present study focused on the interactions between *Bacillus* and yeasts, as well as and *Bacillus* spp. and *L. louembei*. In some cases, it has been observed that when two strains grow together, they regress compared to a single culture (SC). This observation suggests that there is a negative interaction when the two strains coexist, reflecting competition because the two strains compete. Such was the case between *Bacillus* spp. strain VP11 and *S. cerevisiae* strain P3. This kind of interaction occurs when two strains in a medium all consume one or more common nutrients for their growth during fermentation. Competitive interaction for carbon, nitrogen, and iron between yeasts and bacteria isolated from the soil has been demonstrated (Zhou et al., 2022; Arnold, 2022). Likewise, Guo et al. (2020) noted a competing interaction for nutrients between lactic acid bacteria and yeasts, which produce metabolic substances that inhibit their growth together. These harmful substances can be lytic enzymes, antimicrobial peptides. Meng et al. (2015) observed a negative effect of *S. cerevisiae* on the growth of *B. licheniformis* in the fermentation process of Chinese liquor Maotai-flavor. Likewise, some studies have shown that an amensalism interaction between *S. cerevisiae* and *Torulaspora delbruecki*; *S. cerevisiae* normally grows in single and coculture while *T. delbruecki* shows a drop in its growth (Fernandez et al., 2013; Frey-Klett et al., 2011).

In other cases, we have observed a positive effect of the cohabitation of microorganisms, in particular commensalism, as the presence of one stimulates the growth of the other. Our results are similar to those of Fossi et al. (2014), who showed a commensalism interaction between *B. amyloliquefaciens* and *S. cerevisiae* and to those of Sieuwerts et al. (2008) who showed that *Lactobacillus delbrueckii bulgaris* supplied amino acids to *Streptococcus thermophilus* which in turn produced formic acid and CO_2 favorable to growth *L. delbrueckii* subsp. *bulgaris*. Commensalism like interaction has been found in our study between *B. amyloliquefaciens*-*L. louembei*, and *B. pumilus* strain VB15-yeast strain V3 cocultures. This work comes at the right time to give added value to fermented foods in all their diversity.

The importance of studying *Bacillus*-Yeast interactions is a contribution to the understanding of bacteria-bacteria, bacteria-yeast communication; knowing the identity card of the molecules secreted by these microorganisms, means reconsidering the interactions of microorganisms. Mastery of interactomes would enable long-term control of food quality. When communication between microorganisms is disrupted this could lead to the loss of organoleptic characteristics. Good knowledge of molecules such as biosurfactants which are responsible for *Bacillus*-Yeast communication could lead to the development of starters in the sustainable preservation of food because these are the same molecules which play assigned roles in the survival mechanisms of quorum bacteria, sensing and quorum quenching.

Conclusion

The present work demonstrates the microbial grouping in different fermented foods and sheds light on the interactions between *Bacillus* species with *S. cerevisiae* and *L. louembei.* These results will help industries at a national level with the quality control of fermented foods by rationally singling out starters and optimizing their microbiota that are still mischaracterized.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to Mr. Kinouani kinavouidi Duchel Jeandevi, Ms. N'goma-mona Digne Nedjea, Mr. Nkangou Amour Valaire and Ms. Passy Pabie Vincent for their technical support.

REFERENCES

Anjum F, Gautam G, Edgard G, Negi S (2016). Biosurfactant production through *Bacillus s*p. MTCC 5877 and its multifarious applications in food industry. Bioresource Technology 213:262-269.

Arnaouteli S, Bamford NC, Stanley-Wall NR, Kovacs AT (2021). *Bacillus subtilis* biofilm formation and social interactions. Nature Reviews Microbiology 19(9):600-614.

Arnold AE (2022). Bacterial-fungal interactions: Bacteria take up residence in the house that Fungi built. Current Biology 32(7):R327-R328.

Bisht B, Verma M, Sharma R, Chauhan PK, Pant K, Kim H, Vlaskin MS, Kumar V (2023). Development of yeast and microalgae consortium biofilm growth system for biofuel production. Heliyon 9(9):e19353.

Blair KM, Turner L, Winkelman JT, Berg HC, Kearns DB (2008). A molecular clutch disables flagella in the *Bacillus subtilis* biofilm. Science 320(5883):1636-1638.

Cheng F, Tang C, Yang H, Yu H, Chen Y, Shen Z (2013). Characterization of a blend-biosurfactant of glycolipid and lipopeptide produced by *Bacillus subtilis* TU2 isolated from underground oil-extraction wastewater. Journal of Microbiology and Biotechnology

23(3):390-396.
Christensen GD, Simpson WA, Bisno AL, Beachey EH (1982). Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infection and Immunity 37(1):318-326.
Dai J, Dong A, Xiong G, Liu Y, Hossain MS, Liu S, Gao N, Li S, Wang J, Qiu D (2020). Production of Highly Active Extracellular Amylase and Cellulase From *Bacillus subtilis* ZIM3 and a Recombinant Strain With a Potential Application in Tobacco Fermentation. Frontiers in Microbiology 11:1539.
David V, Terrat S, Herzine K, Claisse O, Rousseaux S, Tourdot-Marechal R, Masneuf-Pomarede I, Ranjard L, Alexandre H (2014). High-throughput sequencing of amplicons for monitoring yeast biodiversity in must and during alcoholic fermentation. Journal of Industrial Microbiology and Biotechnology 41(5):811-821.
de Souza Freitas F, Coelho de Assis Lage T, Ayupe BAL, de Paula Siqueira T, de Barros M, Totola MR (2020). *Bacillus subtilis* TR47II as a source of bioactive lipopeptides against Gram-negative pathogens causing nosocomial infections. 3Biotech 10(11):474.
Diaz-Munoz C, Verce M, De Vuyst L, Weckx S (2022). Phylogenomics of a Saccharomyces cerevisiae cocoa strain reveals adaptation to a West African fermented food population. iScience 25(11):105309.
Dymond JS (2013). Preparation of genomic DNA from Saccharomyces cerevisiae. Methods in Enzymology 529:153-160.
Elenga-Wilson PS, Kayath CA, Mokemiabeka NS, Nzaou SAE, Nguimbi E, Ahombo G (2021). Profiling of Indigenous Biosurfactant-Producing *Bacillus Isolates* in the Bioremediation of Soil Contaminated by Petroleum Products and Olive Oil. International Journal of Microbiology 9565930.
Elzeini HM, Ali A, Nasr NF, Hassan M, Hassan AAM, Elenany YE (2021). Probiotic capability of novel lactic acid bacteria isolated from worker honey bees gut microbiota. FEMS Microbiology Letters 368(6).
Eras-Munoz E, Farre A, Sanchez A, Font X, Gea T (2022). Microbial biosurfactants: a review of recent environmental applications. Bioengineered 13(5):12365-12391.
Fernandez de Dios MA, del Campo AG, Fernandez FJ, Rodrigo M, Pazos M, Sanroman MA (2013). Bacterial-fungal interactions enhance power generation in microbial fuel cells and drive dye decolourisation by an ex situ and in situ electro-Fenton process. Bioresource Technology 148:39-46.
Fossi BT, Tavea F, Fontem LA, Ndjouenkeu R, Wanji S (2014). Microbial interactions for enhancement of alpha-amylase production by *Bacillus amyloliquefaciens* 04BBA15 and *Lactobacillus fermentum* 04BBA19. Biotechnology Reports 4:99-106.
Freeman DJ, Falkiner FR, Keane CT (1989). New method for detecting slime production by coagulase negative staphylococci. Journal of Clinical Pathology 42(8):872-874.
Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A (2011). Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiology and Molecular Biology 75(4):583-609.
Guo L, Xu WL, Li CD, Ya M, Guo YS, Qian JP, Zhu JJ (2020). Production technology, nutritional, and microbiological investigation of traditionally fermented mare milk (Chigee) from Xilin Gol in China. Food Science & Nutrition 8(1):257-264.
Irnov I, Winkler WC (2010). A regulatory RNA required for antitermination of biofilm and capsular polysaccharide operons in Bacillales. Molecular Microbiology 76(3):559-575.
Jezierska S, Claus S, Van Bogaert I (2018). Yeast glycolipid biosurfactants. FEBS Letters 592(8):1312-1329.
Jumpathong W, Intra B, Euanorasetr J, Wanapaisan P (2022). Biosurfactant-Producing *Bacillus velezensis* PW192 as an Anti-Fungal Biocontrol Agent against Colletotrichum gloeosporioides and Colletotrichum musae. Microorganisms 10(5).
Kaya-Ongoto MD, Kayath CA, Nguimbi E, Lebonguy AA, Nzaou SAE, Elenga Wilson PS, Ahombo G (2019). Genetic Clearness Novel Strategy of Group I *Bacillus Species* Isolated from Fermented Food and Beverages by Using Fibrinolytic Enzyme Gene Encoding a Serine-Like Enzyme. Journal of Nucleic Acids 5484896.
Kaya-Ongoto MD, Kayath CA, Vouidibio Mbozo AB, Mobandolaka Mitoko G, Elenga Wilson SP, Kinouani Kinavouidi DJ, Nguimbi E (2020). Prime Enzymatic Exocellular Background of *Lysinibacillus* l*ouembei*. Advances in Microbiology 10(3).
Kayath CA, Ibala Zamba A, Mokemiabeka SN, Opa-Iloy M, Elenga Wilson PS, Kaya-Ongoto MD, Mouellet Maboulou RJ, Nguimbi E (2020). Synergic Involvements of Microorganisms in the Biomedical Increase of Polyphenols and Flavonoids during the Fermentation of Ginger Juice. International Journal of Microbiology 8417693.
Kimura K, Yokoyama S (2019). Trends in the application of Bacillus in fermented foods. Current Opinion in Biotechnology 56:36-42.
Konishi M, Nagahama T, Fukuoka T, Morita T, Imura T, Kitamoto D, Hatada Y (2011). Yeast extract stimulates production of glycolipid biosurfactants, mannosylerythritol lipids, by Pseudozyma hubeiensis SY62. Journal of Bioscience and Bioengineering 111(6):702-705.
Marchut-Mikolajczyk O, Drozdzynski P, Polewczyk A, Smulek W, Antczak T (2021). Biosurfactant from endophytic *Bacillus pumilus* 2A: physicochemical characterization, production and optimization and potential for plant growth promotion. Microbial Cell Factories 20(1):40.
Meng X, Wu Q, Wang L, Wang D, Chen L, Xu Y (2015). Improving flavor metabolism of Saccharomyces cerevisiae by mixed culture with *Bacillus licheniformis* for Chinese Maotai-flavor liquor making. Journal of Industrial Microbiology and Biotechnology 42(12):1601-1608.
Mgbodile FC, Nwagu TNT (2023). Probiotic therapy, African fermented foods and food-derived bioactive peptides in the management of SARS-CoV-2 cases and other viral infections. Biotechnol Rep (Amst) 38:e00795.
Mnif I, Besbes S, Ellouze-Ghorbel R, Ellouze-Chaabouni S, Ghribi D (2013). Improvement of bread dough quality by *Bacillus subtilis* SPB1 biosurfactant addition: optimized extraction using response surface methodology. Journal of the Science of Food and Agriculture 93(12):3055-3064.
Ouoba LI, Parkouda C, Diawara B, Scotti C, Varnam AH (2008). Identification of *Bacillus spp.* from Bikalga, fermented seeds of Hibiscus sabdariffa: phenotypic and genotypic characterization. Journal of Applied Microbiology 104(1):122-131.
Ouoba LII, Vouidibio Mbozo AB, Thorsen L, Anyogu A, Nielsen DS, Kobawila SC, Sutherland JP (2015). *Lysinibacillus louembei* sp. nov., a spore-forming bacterium isolated from Ntoba Mbodi, alkaline fermented leaves of cassava from the Republic of the Congo. International Journal of Systematic and Evolutionary Microbiology 65(11):4256-4262.
Pandit S, Fazilati M, Gaska K, Derouiche A, Nypelo T, Mijakovic I, Kadar R (2020). The Exo-Polysaccharide Component of Extracellular Matrix is Essential for the Viscoelastic Properties of *Bacillus subtilis* Biofilms. International Journal of Molecular Sciences 21(18).
Parkouda C, Nielsen DS, Azokpota P, Ouoba LI, Amoa-Awua WK, Thorsen L, Hounhouigan JD, Jensen JS, Tano-Debrah K, Diawara B, Jakobsen M (2009). The microbiology of alkaline-fermentation of indigenous seeds used as food condiments in Africa and Asia. Critical Reviews in Microbiology 35(2):139-156.
Qin Y, Angelini LL, Chai Y (2022). *Bacillus subtilis* Cell Differentiation, Biofilm Formation and Environmental Prevalence. Microorganisms 10(6).
Rajesh R, Gummadi SN (2022). Alpha-Amylase and cellulase production by novel halotolerant *Bacillus sp.*PM06 isolated from sugarcane pressmud. Biotechnol Appl Biochem 69(1):149-159.
Rani M, Weadge JT, Jabaji S (2020). Isolation and Characterization of Biosurfactant-Producing Bacteria From Oil Well Batteries With Antimicrobial Activities Against Food-Borne and Plant Pathogens. Frontiers in Microbiology 11:64.
Schroeckh V, Scherlach K, Nutzmann HW, Shelest E, Schmidt-Heck W, Schuemann J, Martin K, Hertweck C, Brakhage AA (2009). Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in Aspergillus nidulans. Proceedings of the National Academy of Sciences 106(34):14558-14563.
Sieuwerts S, de Bok FA, Hugenholtz J, van Hylckama Vlieg JE (2008). Unraveling microbial interactions in food fermentations: from classical to genomics approaches. Applied and Environmental Microbiology 74(16):4997-5007.
Stancu MM (2020). Biosurfactant production by a *Bacillus megaterium* strain. Open Life Sciences 15(1):629-637.
Wu B, Xiu J, Yu L, Huang L, Yi L, Ma Y (2022). Biosurfactant production by *Bacillus subtilis* SL and its potential for enhanced oil recovery in

low permeability reservoirs. Scientific Reports 12(1):7785.
Xu H, Zou Y, Lee HY, Ahn J (2010). Effect of NaCl on the biofilm formation by foodborne pathogens. Journal of Food Science 75(9):M580-585.
Zara G, Budroni M, Mannazzu I, Fancello F, Zara S (2020). Yeast biofilm in food realms: occurrence and control. World Journal of Microbiology and Biotechnology 36(9):134.
Zhou Y, Wang H, Xu S, Liu K, Qi H, Wang M, Chen X, Berg G, Ma Z, Cernava T, Chen Y (2022). Bacterial-fungal interactions under agricultural settings: from physical to chemical interactions. Stress Biology 2(1):22.

Vol. 17(12), pp. 306-313, December 2023
DOI: 10.5897/AJMR2023.9728
Article Number: A5FD8A671642
ISSN: 1996-0808
Copyright©2023
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Prevalence, antibiotic resistance of *Staphylococcus aureus* isolated from layer farms (eggs, droppings/litter) in the outskirts of the city of Ouagadougou, Burkina Faso

Abdallah Sawadogo[1]*, Assèta Kagambèga[1], Daniel Soro[1], Henseni Bognini[1], Michel Dione[2], Haoua Cissé[1], Ibonyé Dieni[1] and Nicolas Barro[1]

[1]Department of Biochemistry-Microbiology, Doctoral School of Science and Technology, Joseph KI-ZERBO University, Ouagadougou 03 BP 7021, Burkina Faso.
[2]International Livestock Research Institute, Dakar BP 24265, Senegal.

Received 19 October, 2023; Accepted 1 December, 2023

Eggs are susceptible to microbiological contamination by various pathogens on farms, particularly those belonging to the genus *Staphylococcus*, which are zoonotic bacteria capable of contaminating humans, animals, and/or birds, posing a significant public health concern. In Burkina Faso, limited data exists on the prevalence of *Staphylococcus aureus*, prompting this study to investigate the prevalence and antibiotic resistance of *S. aureus* on layer farms. To achieve this, a socio-demographic survey was conducted, followed by egg sampling from different farms. A pool of six eggs constituted one sample for each farm, and microbiological analysis was performed using standard methods. The antibiotic susceptibility of the strains was also tested. The results indicated a prevalence of 51.78% from litter, 35.59% from shells, and 10.16% from egg contents. The strains exhibited the highest resistance levels to Tetracycline (98%), Erythromycin (84%), Trimethoprim/Sulfamethoxazole (77%), Colistin (56%), Oxacillin (55%), and Cefoxitin (38%). However, the strains showed a high sensitivity to gentamycin (81%), and 56% were sensitive to Chloramphenicol. These findings highlight the contamination of eggs by antibiotic-resistant *S. aureus*, emphasizing the need to enhance good practices in the egg production chain to prevent such contamination.

Key words: Prevalence, antibiotic resistance, *Staphylococcus aureus*, poultry, Ouagadougou.

INTRODUCTION

According to FAO (2019), 1.6 million households (56%) engage in poultry farming in Burkina Faso, with intensive farms predominantly located around major cities. The breeding of imported chicks, particularly laying hens, is flourishing due to the high demand for eggs in various cities. Table eggs serve as a vital protein source for

*Corresponding author. E-mail: abdallahsawadogo11@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

vulnerable populations, and in some cases, they are utilized in the treatment and recovery of Malnourished children (Stark et al., 2021). Despite eggs being considered a complete food for growth and sustenance, they are susceptible to contamination by various microorganisms (Buriro et al., 2017).

Staphylococcus species are among the microflora found on the surface of table eggs, posing a risk of spoilage and infection for consumers (Salihu et al., 2015). Eggshells harbor numerous microorganisms, including *Staphylococcus* spp., *Salmonella* species, *Streptococcus* species, *Escherichia coli, Bacillus* species, and *Listeria monocytogenes* (Mahdavi et al., 2012). Common foodborne pathogens on eggshells include *E. coli and S. aureus* (Maktabi et al., 2018). *Staphylococcus* spp. are zoonotic pathogenic bacteria, capable of contaminating humans, animals, and birds, contributing to the emergence of zoonotic pathogens that infect domestic and wild animals (Dowd et al., 2013).

Methicillin-resistant *S. aureus* (MRSA) has been identified by the World Health Organization as a high-priority pathogen urgently requiring new antibiotics (WHO, 2017).

S. aureus infections range from superficial skin infections to life-threatening syndromes, including subcutaneous abscess, impetigo, osteomyelitis, infective endocarditis, pneumonia, septicemia, and septic shock syndrome (Gordon and Lowy, 2008). While studies in Burkina Faso on poultry have highlighted the presence of pathogenic bacteria with high antimicrobial resistance in poultry and poultry products (Kagambèga et al., 2018; Bouda et al., 2019), there is currently no available data on the prevalence of *S. aureus* in eggs. Consequently, monitoring and controlling the circulation of *S. aureus* in entities such as humans, animals, and the environment are imperative. The lack of data on the prevalence of *S. aureus* in eggs in Burkina Faso is a significant concern, prompting this study to assess the prevalence and antibiotic resistance of *S. aureus* in eggs and the associated risk practices in egg farming.

MATERIALS AND METHODS

Ethical clearance

The study received approval from the ethical committee of the Ministry of Health, Burkina Faso, under reference number 2020-9-186. Informed consent was obtained from each participant before they were interviewed, and consequently, all participants provided their consent to participate in the study.

Study areas and sampling

A cross-sectional study was conducted between February and May, 2022 in urban and peri-urban poultry farms in Ouagadougou, the most densely populated city with 2,415,266 inhabitants (INSD, 2022). Given the high demand for meat and eggs, poultry farmers have established their operations in and around the city to meet this demand.

Data collection and sampling

A socio-demographic survey was conducted through direct interviews with stakeholders on the farms. Following the compilation of survey data, sampling was performed on the selected farms. For litter, fifty-six samples were collected (from five different points to form one sample from each farm). In the case of egg sampling, a sample of ten eggs was collected from each farm. Once at the laboratory for analysis, six eggs were chosen from the ten to form a sample (pooling of six), resulting in a total of fifty-nine egg samples taken from an equal number of farms. All samples were labeled, placed in freezer bags, stored in coolers containing ice, and transported to the laboratory for microbiological analysis.

Microbiological analysis

Culture media were prepared following the manufacturer's instructions, and microbiological analysis was conducted on a sample of six eggs from each farm. The shell was examined for germs using shell rinsing water, utilizing the method described by Moats (1981). Each sample (a pool of 6 eggs) was washed in a sterile Stomacher bag with 100 ml buffered peptone water (Liofilchem, Italy). 10 µL of this solution was inoculated onto Baird Parker medium supplemented with egg yolk and tellurite. After rinsing, the eggs were disinfected with bleach, alcohol, and dried. The eggs were then prepared for evacuation of their contents according to the procedure outlined by APHA (2004). The eggs were cracked with a sterile rod, and the contents were poured into a Stomacher bag. The contents were mixed and agitated in the Stomacher bag. A quantity of 10 ml was taken and introduced into 90 ml of peptone-buffered water, and 10 µL of this solution was seeded onto Baird Parker medium.

For litter analysis, 10 g of each sample was weighed and dissolved in 90 ml of buffered peptone water, and 10 µL was inoculated into Baird Parker medium. The isolation and identification of coagulase-positive *S. aureus* were carried out following ISO (2003) standards. Germ identification involved microscopic observation, Gram staining, catalase, and coagulase positive tests of suspect colonies. Strains confirmed after identification (109) were tested for antibiotic susceptibility in accordance with EUCAST (CA-SFM /EUCAST, 2022), using *S. aureus* ATCC 29213 as a reference strain.

The antibiotics chosen were those commonly used locally. The disk diffusion method was employed for all antibiotics except colistin, for which the liquid dilution method was used. Furthermore, methicillin-resistant *S. aureus* (MRSA) strains were phenotypically identified when the diameter of the cefoxitin disc (30 µg) was ≤21 mm (CLSI, 2020). The most common antibiotics were selected to assess the susceptibility of the strains, including Chloramphenicol (C), Ciprofloxacin (CIP), Gentamycin (CN), Colistin (CT), Erythromycin (E), Cefoxitin (FOX), Oxacillin (OX), Trimethoprim-Sulfamethoxazole (SXT), Tetracycline (TE), and Vancomycin (VA).

RESULTS

The characteristics of those interviewed are shown in Table 1. The respondents comprised 74% male and 36% female participants. The predominant educational level was secondary school (46%), followed by university education (26%). A notable 19% of respondents had received no formal education. Only 40% of the participants had undergone training in livestock farming before commencing their activities. Regarding knowledge of microbiology, 79% indicated having little knowledge of bacteria.

Table 1. Socio-demographic characteristics.

Characteristics	Category	Effective (%)
Sex	Man	52 (74)
	Woman	18 (36)
Education level	No formal education	13 (19)
	Primary	7 (10)
	Secondary	32 (46)
	Academic	18 (26)
Training	Vocational training in animal husbandry	28 (40)
	No training related to breeding	42 (60)
Notion about bacteria	Yes	55 (79)
	No	15 (21)

Farm characteristics and practices

The poultry breeds with the highest numbers were Dutch Blue at 40%, Local Improved at 26% and Isa Brown at 23%. Chickens were primarily housed day and night (79%). Concerning hygiene on the farms, the cleaning methods applied included standard cleaning, involving the cleaning and removal of waste/droppings, along with the replacement of shavings. In terms of cleaning frequency, 46% of respondents cleaned monthly, 21% cleaned every two weeks, and 21% cleaned when droppings became heavy on the farm. Additionally, 56% used disinfectant or soapy water for cleaning. Finally, 94% did not observe a waiting period after drug administration before resuming normal production use. Table 2 shows the farm characteristics and practices.

Antibiotics use in farms

The antibiotics used on the farms in the 4 weeks prior to the study are as shown in Figure 1. The most used antibiotic family is Tetracycline (67.1%), followed by Macrolides (19.3%).

A total of four hundred and twenty-seven suspect colonies were isolated. After confirmatory tests, one hundred and nine colonies were confirmed as coagulase-positive *S. aureus* strains. The prevalences of *S. aureus* in the matrices analyzed are shown in Table 3. The highest prevalence was observed in the litter sample with 51.78% (29/56). Eggshell showed a prevalence of 35.56% (21/59), and the prevalence for egg contents was 10.16% (06/59).

Antibiotic resistance

S. aureus strains exhibited high resistance to antibiotics such as Tetracycline (98%), Erythromycin (84%), Trimethoprim/Sulfamethoxazole (77%), Vancomycin (59%), Colistin (56%), Cefoxitin, and Oxacillin (55%, 38%).

Conversely, these strains demonstrated sensitivity to Gentamicin (81%) and Chloramphenicol (56%). Additionally, 79% of strains showed intermediate resistance to Ciprofloxacin (Figure 2).

DISCUSSION

The study reveals that 94% of respondents do not adhere to waiting times after drug prescriptions, a rate higher than the 56.66% reported by Samandoulougou et al. (2016). This discrepancy may be attributed to the nature of the farms under consideration in this study (layers). Adhering to waiting times would involve classifying all eggs as non-saleable during this period, leading to financial losses for the farmers. Non-compliance with waiting periods poses a risk of food product contamination by drug residues, which could result in intoxication, allergies, and antibiotic resistance (Goff et al., 2017).

The survey indicated that the most common cleaning method involved the superficial removal of residues and feces every month without disinfectants. Regarding cleaning eggs after collection, only 19% used water for rinsing before sale, and 81% relied solely on their hands to clean the surface of the eggs. This might explain the presence of feces on the shell in 2% of cases. These hygiene deficiencies could contribute to the contamination and spread of pathogens.

The results demonstrate a high resistance to Tetracyclines (98%) among the study strains, potentially explained by their widespread use and affordable cost in animal health. This aligns with Figure 1, which indicates that 67.1% of respondents used Tetracyclines on their farms. The study suggests that antimicrobial resistance

Table 2. Farm characteristics and practices.

	Category	Effective (%)
Chicken breed	Dutch blue	28 (40)
	Improved local chicken	18 (26)
	Isa Brown	16 (23)
	Other	8 (11)
Chicken keeping	Housed day and night	55 (79)
	Free by day and housed by night	7 (10)
	Freedom day and night	8 (11)
Farm cleaning	Standard cleaning	58 (83)
	Copal replacement	12 (17)
Use of medication without professional advice	Drugs already used without professional advice	51 (73)
	Always after professional advice	19 (27)
Waiting time after drug administration	Not respecting waiting periods	66 (94)
	Respecting waiting times	4 (6)
Cleaning frequency	Every day	9 (13)
	Chips changed every two weeks	15 (21)
	Every month	32 (46)
	Every 3 months	4 (6)
	Whenever there are too many droppings	15 (21)
	Every 6 months	1 (1)
Use of disinfectants to clean farm	No disinfectants	31 (44)
	Veterinary disinfectant	25 (36)
	Bleach	12 (17)
	Soap	2 (3)
Hygiene appreciation	Satisfied	10 (14)
	Acceptable	27 (39)
	Insufficient*	33 (47)
Egg cleaning	Rinse with cloth or by hand	57 (81)
	Disinfectant solution (soapy water or bleach)	13 (19)

*In 2% of cases, pieces of feces and feathers were found on the shell.

(AMR) of livestock origin is increasing in low- and middle-income countries (LMICs), particularly in poultry and pigs (Van Boeckel et al., 2019). A more recent study has demonstrated that antibiotic use in poultry farms significantly increases resistance in bacterial strains present in the upper layer of soil around the poultry farm within at least a 25 m range (Kousar et al., 2021). The prevalence of *S. aureus* in egg content and eggshell was 8.75 and 34.46%, respectively, in the present study. The prevalences are lower than the 13.3 and 40% reported in egg content and eggshell, respectively, in Egypt by Sadek et al. (2016). The presence of microorganisms in eggs could be attributed to the fact that the egg leaves the hen's body through the same passage as feces are excreted. *S. aureus* contamination of eggs is a major concern, especially considering that eggs are consumed by immunocompromised individuals and malnourished children. The contamination of the eggshell and contents might be associated with the type of floor-laying farm where the eggs come into contact with droppings (Gunnarsson et al., 2020). Frequent cleaning of the farm could reduce contact between eggs and droppings and

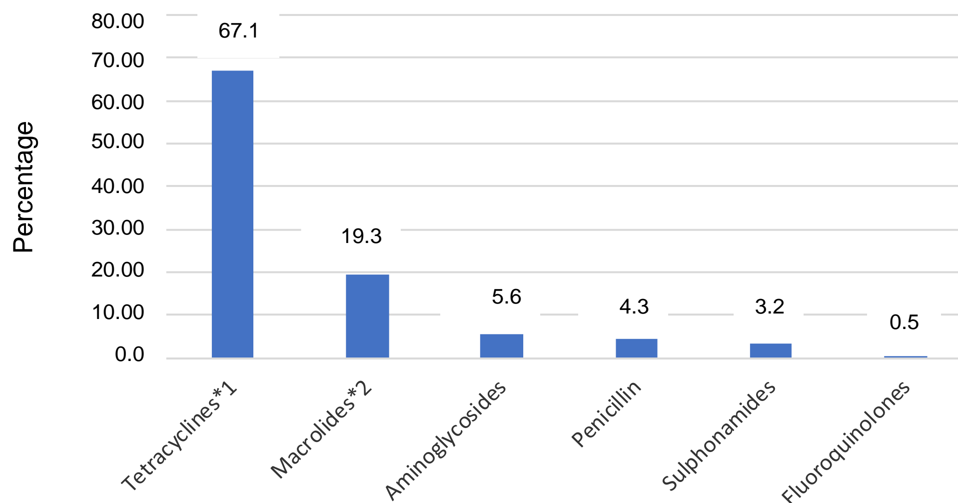


Figure 1. Antibiotics used in the last 4 weeks preceding survey. Tetracycline*1: Among the farmers who used tetracyclines (47/70), 85.1% (40/47) used them as a preventive measure. Macrolides *2: Among farmers who used macrolides (14/70), 92.85% (13/14) used them preventively.

Table 3. Prevalence of S. aureus by sampling.

Sample	Prevalence of *S. aureus*
Litter (n= 56)	29/56 (51.78%)
Shell (n= 59)	21/59 (35.59%)
Egg content (n= 59)	06/59 (10.16%)

n: Number of sample.

dust, thereby limiting egg contamination.

Microorganisms present inside an intact or uncracked egg may be due to the presence of a pathogen in the hen's ovary or oviduct before the shell forms around the yolk and albumen (USDA FSIS, 2011). Ansah et al. (2009) have reported that, as eggs are left for longer periods, their resistance decreases, allowing organisms to penetrate the egg contents. Warm weather could facilitate the penetration of *S. aureus* present on the egg surface by weakening the shell.

Storing eggs at room temperature without refrigeration is a factor that could permit the proliferation of germs in eggs (Momani et al., 2017), explaining the prevalence of *S. aureus* in eggs in this present study.

A study carried out on layers in Pakistan showed that daily cleaning of drinking troughs helped reduce the bacterial load in layers. This suggests that both the flock of birds and consumers of eggs and chicken meat are at risk of bacterial infection if strict farm hygiene is not ensured through regular monitoring (Folorunso et al., 2014).

According to a study on eggs by El-Toukhy et al. (2021) in Egypt, *S. aureus* exhibited 90% resistance to Ciprofloxacin, which is significantly higher compared to the resistance reported in this study (20%). Additionally, these authors reported 65% resistance to Erythromycin and 100% resistance to Trimethoprim-Sulfamethoxazole and Tetracycline. The resistance to Tetracycline is similar to that reported in the present study (98%). Tetracycline and Chloramphenicol resistance are also similar to that reported in poultry eggs by Buriro et al. (2017) (100%). These elevated levels of resistance could be attributed to the strains' exposure to antibiotics, as a previous study revealed the misuse of antibiotics in poultry farms (CDC, 2019). Antibiotic-resistant bacteria isolated from these samples can be transmitted to humans through handling and ingestion of raw eggs or egg products. Given that eggs are a vital protein source, especially for vulnerable populations, such as cases where eggs are used for the treatment and recovery of malnourished children (Stark et al., 2021); these strains pose a significant risk to consumers, especially the most vulnerable, such as children and immunocompromised individuals.

S. aureus strains isolated from the nasopharynxes of children in Burkina Faso showed over 85% resistance to Tetracycline and 96% resistance to Penicillin (Bonko et

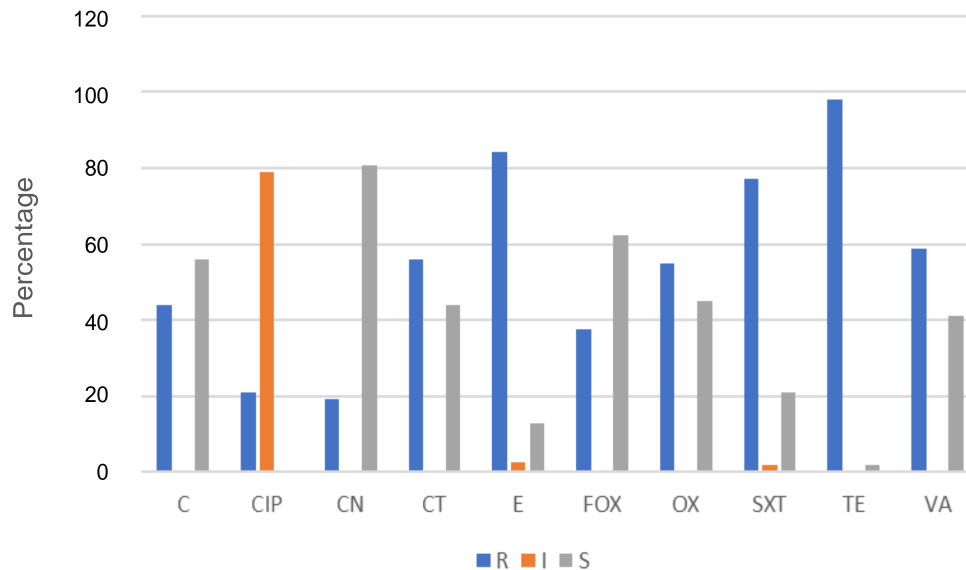


Figure 2. Susceptibility of *S. aureus* strains to antibiotics. R: Resistant; I: Intermediate; S: Sensible, C: Chloramphenicol; CIP: Ciprofloxacin; CN: Gentamycin; CT: Colistine, E: Erythromycin; FOX: Cefoxitine; OX: Oxacilline; SXT: Triméthoprime-Sulphametoxazole; TE: Tetracycline; VA: Vancomycin.

al., 2021). Other *S. aureus* strains isolated from nasal carriage in patients in Burkina Faso exhibited high rates of resistance, with amoxicillin at 90%, followed by tetracycline at 61%, and erythromycin at 54% (Ouedraogo et al., 2016), confirming the trend in *S. aureus* antibiotic resistance. Vancomycin resistance was detected in 59% of the tested isolates, a rate lower than the 70% reported by El-Toukhy et al. (2021). This represents a considerably high rate of resistance, raising concerns as Vancomycin is the drug of choice for treating MRSA. Resistance to Cefoxitin was 38%, indicating a phenotypic MRSA prevalence of 38%. Antibiotics are extensively used for preventive purposes on a large scale (Figure 1), contributing to increased antibiotic resistance in the relevant germs (WHO, 2021). The observed resistance to oxacillin (55%) is lower than that reported by Syed et al. (2019) (83.6%). Oxacillin is a molecule more commonly used in humans, so the relatively high resistance observed in poultry may be attributed to transfer from humans to the farm through various contacts, such as human feces discharged in nature or direct contact between humans and the farm (Syed et al., 2019). MRSA contamination poses significant risks to human health, particularly for individuals living and working on farms (da Silva et al., 2020). Infections caused by MRSA result in higher expenses in the public health sector and a greater morbidity and mortality rate compared to non-resistant strains (Guzmán-Blanco et al., 2009).

Poultry manure, often used as organic fertilizer in agriculture without prior treatment, contains antibiotic residues that persist in the environment for an extended period. These residues can eventually reach the human body through contaminated food or crops. According to the Food and Agriculture Organization (FAO), the persistence of antibiotics in the environment could contribute to the spread of antibiotic residues, likely leading to resistance or even allergies after the ingestion of contaminated food (FAO, 2008). Immersing eggs in disinfectants (H_2O_2 solution) could reduce the microbial load on eggs before commercialization (El-Toukhy et al., 2021). The use of an easily available natural product, baking soda, could provide a low-cost decontamination solution while protecting the environment from chemical pollution (Fong et al., 2011).

Conclusion

This study revealed a lack of hygiene in various stages of the egg production chain, potentially contributing to the presence of *S. aureus* in farms, on eggshells, and in egg contents. Among these bacterial strains, a high level of antibiotic resistance was observed against several antibiotics, and multi-resistant bacteria were also identified. The circulation of these resistant strains on farms poses a major health concern, particularly considering that eggs are consumed by even the most vulnerable populations. It is imperative to prioritize and adhere to hygienic measures in egg production. Additionally, there is a pressing need to regulate the use of antibiotics and implement measures to control the circulation of resistant bacteria on farms.

CONFLICT OF INTERESTS

The authors have not declared ant conflict of interests.

REFERENCES

Ansah T, Dzoagbe GSK, Teye GA, Adday S, Danquah JK (2009). Microbial quality of beef sold on selected markets in the Tamale Metropolis in the Northern Region of Ghana. Livestock Research for Rural Development 23(1):1-8.

American Public Health Association (APHA) (2004). Standard methods for the examination of dairy products. 17th Ed. American Public Health Association. Washington D.C.

Van Boeckel TP, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, Gilbert M, Bonhoeffer S, Laxminarayan R (2019). Global trends in antimicrobial resistance in animals in low- and middle-income countries. Science 365(6459):1-5. Available at: https://doi.org/10.1126/science.aaw1944.

Bonko MDA, Lompo P, Tahita MC, Kiemde F, Karama I, Somé AM, Mens PF, Menting S, Tinto H, Schallig HDFH (2021). Antibiotic susceptibility of staphylococcus aureus and streptococcus pneumoniae isolates from the nasopharynx of febrile children under 5 years in Nanoro, Burkina Faso', Antibiotics 10(4). Available at: https://doi.org/10.3390/antibiotics10040444.

Bouda SC, Kagambèga A, Bonifait L, Gall FL, Bawa HI, Bako E, Bagre TS, Zongo C, N'diaye AW, Traore SA, Chemaly M, Salvat G, Barro N (2019). Prevalence and Antimicrobial Resistance of Salmonella enterica Isolated from Chicken and Guinea Fowl in Burkina Faso. International Journal of Microbiology and Biotechnology 4(3):64-71.

Buriro RS, Habib F, Rind R, Shah MA, Kaleri RR, Samoon AK, Talpur MA, Goswami N (2017). Antibiotic sensitivity tests of various micro-organisms from poultry eggs. Pure and Applied Biology 6(4):1417-1426. Available at: https://doi.org/10.19045/bspab.2017.600153.

Center for Disease Control (CDC) (2019*).* Antibiotic Resistance Threats Report | CDC. Center for Disease Control. Published 2021. Accessed 27 November 2023. https://www.cdc. gov/drugresistance/Biggest-Threats.html'.

Clinical and Laboratory Standards Institute (CLSI) (2020). Performance standards for antimicrobial susceptibility testing; a CLSI supplement for global application (Wayne, PA 19087 USA: Clinical and Laboratory Standards Institute 950 West Valley Road, Suite 2500), 332. CLSI document M02, M07, and M11.

CA-SFM /EUCAST (2022). Comité de l 'antibiogramme de la Société Française de Microbiologie. Société Française de Microbiologie, *1.1*:181.

Da Silva AC, Rodrigues MX, Silva NCC (2020). Methicillin-resistant Staphylococcus aureus in food and the prevalence in Brazil: a review. Brazilian Journal of Microbiology 51(1):347-356. Available at: https://doi.org/10.1007/s42770-019-00168-1.

Dowd K, Taylorb M, Toribioa JALML, Hookerc C, Dhand NK (2013). Zoonotic disease risk perceptions and infection control practices of Australian veterinarians: Call for change in work culture. Preventive Veterinary Medicine 111(1-2):17-24. Available at: https://doi.org/10.1016/j.prevetmed.2013.04.002.

El-Toukhy EI, Hefny EG, Koraney NF, Azam AE (2021). Eggshell contamination with special reference to different methods of disinfection on the isolated bacteria. Benha Veterinary Medical Journal 41(1):13-18. Available at: https://doi.org/10.21608/bvmj.2021.76826.1414.

Food and Agriculture Organization (FAO) (2008). La biosécurité au service de la lutte contre l'influenza aviaire hautement pathogène : contraintes et solutions poss*ibles*. Rome.

Food and Agriculture Organization (FAO) (2019). Le devenir de l'élevage au Burkina Faso. Défis et opportunités face aux incertitudes. Rome.

Folorunso OR, Kayode S, Onibon VO (2014). Poultry farm hygiene: microbiological quality assessment of drinking water used in layer chickens managed under the battery cage and deep litter systems at three poultry farms in Southwestern Nigeria. Pakistan Journal of Biological Sciences 17(1):74-79. Available at: https://doi.org/10.3923/pjbs.2014.74.79.

Fong D, Gaulin C, Lê ML, Shum M (2011). Effectiveness of Alternative Antimicrobial Agents for Disinfection of Hard Surfaces, September pp. 1-19.

Goff DA , Kullar R, Goldstein EJC, Gilchrist M, Nathwani D, Cheng AC, Cairns KA, Escandón-Vargas K, Villegas MV, Brink A, Bergh DVD, Mendelson M (2017). A global call from five countries to collaborate in antibiotic stewardship: united we succeed, divided we might fail'. The Lancet Infectious Diseases 17(2):e56-e63. Available at: https://doi.org/10.1016/S1473-3099(16)30386-3.

Gordon RJ, Lowy FD (2008). Pathogenesis of methicillin-resistant Staphylococcus aureus infection. Clinical Infectious Diseases 46:5. Available at: https://doi.org/10.1086/533591.

Gunnarsson S, Segerkvist KA, Wallgren T, Hansson H, Sonesson U (2020). A systematic mapping of research on sustainability dimensions at farm-level in pig production'. Sustainability (Switzerland) 12(11):1-17. Available at: https://doi.org/10.3390/su12114352.

ISO Standard DIS 6888 (2003). (E): Horizontal method for the enumeration of Coagulase Positive *Staphylococci (Staphylococcus aureu*s and other species). https://www.iso.org/standard/33147.html

Kagambèga A, Thibodeau A, Trinetta V, Soro DK, Sama FN, Bako E, Bouda CS, Wereme A, N DiayeP, Fravalo N (2018). Salmonella spp . and Campylobacter spp . in poultry feces and carcasses in Ouagadougou, Burkina Faso', pp. 1-6. Available at: https://doi.org/10.1002/fsn3.725.

Kousar S, Rehman N, Javed A, Hussain A, Naeem M, Masood S, Ali HA, Manzoor A, Khan AA, Akrem A, Iqbal F, Zulfiqar A, Jamshaid MB, Waqas M, Waseem A, Saeed MQ (2021). Intensive poultry farming practices influence antibiotic resistance profiles in pseudomonas aeruginosa inhabiting nearby soils. Infection and Drug Resistance 14:4511-4516. Available at: https://doi.org/10.2147/IDR.S324055.

Mahdavi M, Jalali M, Safaei HG, Shamloo E (2012). Microbial quality and prevalence of Salmonella and Listeria in eggs'. International Journal of Environmental Health Engineering 1(1):48. Available at: https://doi.org/10.4103/2277-9183.105347.

Maktabi S, Zarei M, Rashnavady R (2018). Effect of sequential treatments with sodium dodecyl sulfate and citric acid or hydrogen peroxide on the reduction of some foodborne pathogens on eggshell. Iranian Journal of Veterinary Research 19(2):113-117.

Guzmán-Blanco M, Mejía C, Isturiz R, Alvarez C, Bavestrello L, Gotuzzo E, Labarca J, Luna CM, Rodríguez-Noriega, Salles MJC, Zurita CSJ (2009). Epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) in Latin America 34(4):304-308. Available at: https://doi.org/10.1016/j.ijantimicag.2009.06.005.

Moats WA (1981). Factors Affecting Bacterial Loads on Shells of Commercially Washed Eggs. Poultry Science 60(9):2084-2090. Available at: https://doi.org/10.3382/ps.0602084.

Momani WA, Janakat S, Khatatbeh M (2017). Bacterial contamination of table eggs sold in Jordanian markets. Pakistan Journal of Nutrition 17(1):15-20. Available at: https://doi.org/10.3923/pjn.2018.15.20.

National Institute of Statistics and Demography (INSD) (2022). Fifth General Census ofPopulation and Housing Presentation ofthe Final Results; National Institute of Statistics and Demography (INSD): Ouagadougou, Burkina Faso, p. 136. Available at: https://www.finances.gov.bf/fileadmin/user_upload/storage/Resultats_definitifs_RGPH_2019.pdf.

Ouedraogo AS, Dunyach-Remy C, Kissou A, Sanou S, Poda A, Kyelem CG, Solassol J, Bañuls AL, Van De Perre, P, Ouédraogo R, Jean-Pierre H, Lavigne JP, Godreuil S (2016). High Nasal Carriage Rate of Staphylococcus aureus Containing Panton-Valentine leukocidin- and EDIN-Encoding Genes in Community and Hospital Settings in Burkina Faso. Frontiers in Microbiology 7:1406. Available at: https://doi.org/10.3389/fmicb.2016.01406.

Sadek OA, Hussein MF, Berbawy SMEL (2016). Microbiological Status of Farms and Baladi Hens' Eggs. Assiut Veterinary Medical Journal 62(148):58-68. Available at: https://doi.org/10.21608/avmj.2016.169213.

Salihu MD, Garba B, Isah Y (2015). Evaluation of microbial contents of table eggs at retail outlets in Sokoto metropolis, Nigeria. Sokoto Journal of Veterinary Sciences 13(1):22-28.

Samandoulougou S, Ilboudoo AJ, Sanon G, Tindano K, Compaoré H, Ouédraogo A Savadogo A, Traoré AS (2016). Utilisation de medicaments veterinaires en production bovine et aviaire et securite sanitaire des aliments d'origine animale AU BURKINA FASO. The International Journal of Multi-disciplinary Sciences 1(4):50-79.

Stark H, Omer A, N'Diaye AW, Sapp AC, Moore EV, McKune SL (2021). 'The Un Oeuf study: Design, methods and baseline data from a cluster randomised controlled trial to increase child egg consumption in Burkina Faso'. Maternal and Child Nutrition 17(1):1-13. Available at: https://doi.org/10.1111/mcn.13069.

Syed MA, Jackson CR, Ramadan H, Afridi R, Bano S, Bibi S, Fatima B, Tabassum S, Jamil B, Khan MF, Barrett JB, Woodley TA (2019). Detection and Molecular Characterization of Staphylococci from Eggs of Household Chickens. Foodborne Pathogens and Disease 16(8):550-557. Available at: https://doi.org/10.1089/fpd.2018.2585.

USDA FSIS (2011). Shell eggs from farm to table. Available at: www.fsis.usda.gov.

World Health Organization (WHO) (2017). News. Available at: https://www.who.int/fr/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

World Health Organization (WHO) (2021). Antimicrobial Resistance. Available at: https://www.who.int/news-room/fact-sheets/detail/antimicrobial- resistance (accessed on 27 November 2023).

Vol. 17(12), pp. 314-319, December 2023
DOI: 10.5897/AJMR2023.9723
Article Number: DFCDF3F71650
ISSN: 1996-0808
Copyright©2023
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Acute bacterial meningitis ecosystem in Cote D'ivoire from 2012 to 2020

Sophia Chiayé Okpo[1]*, Clarisse Kouamé Elogne[1], Tatiana Asseh Kangah[1], Jean Claude Blessa Anné[1], Kouadio N'guessan[1], Solange Kakou-Ngazoa[2] and Mireille Dosso[1,2]

[1]National Center of Reference of Meningitis, Institut Pasteur de Côte d'Ivoire, 01 BP 490, Abidjan, Côte d'Ivoire.
[2]Molecular Biology Platform, Institut Pasteur de Côte d'Ivoire, 01 BP 490, Abidjan, Côte d'Ivoire.

Received 27 September, 2023; Accepted 21 November, 2023

Bacterial meningitis remains a health concern in meningitis belt countries. The objective of this study was to ascertain the bacterial profiles of meningitis in Côte d'Ivoire from 2012 to 2020. The study, conducted retrospectively at the National Center of Reference of Meningitis of Cote d'ivoire, analyzed 5046 cerebrospinal fluid (CSF) samples from January 2012 to December 2020. Epidemiological data were collected as part of the study. All the samples were subjected to analysis using classic bacteriology, antibiogram, and real-time polymerase chain reaction (PCR) methods. More than 80% of the studied population fell within the age range of 0 to 5 years. The male-to-female sex ratio was 1.45, translating to 2987 men and 2059 women. *Neisseria meningitidis* was the predominant pathogen in 2012 (59%) and 2013 (64%). However, from 2014 to 2020, *Streptococcus pneumoniae* became the most frequently identified pathogen, ranging from 44.74 to 77.30%. *Haemophilus influenzae* type b was observed from 2015 to 2020, with prevalence rates ranging from 14.3 to 16.66%. *H. influenzae* non-b was observed with rates ranging from 10 to 15% from 2018 to 2020. No cases of *N. meningitidis* A were reported. The emergence of bacteria responsible for meningitis, such as pneumococcus and *H. influenzae*, was noted. Microbiological monitoring is deemed essential for the prevention of meningitis.

Key words: Emergence,bacterial, meningitis, ecosystem, monitoring.

INTRODUCTION

Bacterial meningitis remains a significant health concern in meningitis belt countries (Diallo et al., 2017). The highest incidences (per 100,000) were reported in Niger (7.71 in 2015) and Burkina Faso (10.2 in 2012) (Pardo de Santayana et al., 2023). The primary agents responsible for bacterial meningitis include *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae* b (Kyaw et al., 2002; Gervaix, 2014; Mamadou et al., 2019). In Niger, for instance, *N. meningitidis* accounted for the highest disease burden, with 4741 (84.8%) confirmed cases, compared to 721 cases of *S. pneumoniae* (12.9%) and 129 cases of *H. influenzae* (2.3%) (Sidikou et al., 2019). These microorganisms, including *S. pneumoniae* (Gram-positive capsulated lanceolate diplococci, or in a candle flame), *N. meningitidis* (a Gram-negative cocci resembling coffee beans), and *H. influenzae* (a bacillus or cocobacillus), are fastidious and thrive in an atmosphere enriched in CO_2

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

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

when cultivated on blood-enriched media. They form part of the normal flora of the mucous membranes in the upper respiratory tracts of both children and adults. Colonization typically commences shortly after birth and persists throughout an individual's life. These bacteria are strictly human pathogens, spreading through the air or direct contact among children or adults via Pflugge droplets. Therefore, when alterations of the mucosa, along with other contributing factors, facilitate the colonization and subsequent multiplication of various bacterial species, infections such as otitis, pneumonia, septicemia, and meningitis can occur (Medical Microbiology Course, 2023).

Approximately, half of all cases are reported in sub-Saharan Africa during the dry season, particularly in an area referred to as the "meningitis belt" (Lapeyssonnie, 1963). In Côte d'Ivoire, meningitis is endemic in two distinct epidemiological zones. Epidemics of meningitis caused by *N. meningitidis* serogroups A, Y, and W have been observed, similar to trends in other countries within this epidemiological zone. In the southern part of the country, which is situated in a humid tropical zone, meningitis occurs sporadically, with serogroup C being predominant. The introduction of the *N. meningitidis* A vaccine has led to a shift in the bacteriological profile of meningitis. In Burkina Faso, for example, *S. pneumoniae* has become the predominant pathogen, accounting for 57%, followed by *N. meningitidis* at 40%, with serogroup W being the most prevalent, and *H. influenzae* at 2% (Diallo et al., 2017). Simonetta's (2022) findings revealed that serogroup A meningococcal disease had disappeared from all age groups in many countries within the meningitis belt. Certainly, Côte d'Ivoire introduced the meningococcal A conjugate vaccine in 2010, along with the *H. influenzae* b vaccine and the pneumococcal conjugate vaccine as part of the Expanded Program on Immunization (EPI). As in other countries, the introduction of these vaccines prompts the question of whether it has contributed to a shift in the microbiological profile. Microbiological monitoring of acute meningitis stands as a crucial component of enhanced surveillance. The objective of this study was to ascertain the bacterial profiles of meningitis in Côte d'Ivoire from 2012 to 2020.

MATERIALS AND METHODS

Study design and sampling

This retrospective cross-sectional study utilized data collected at the National Center of References for Meningitis (NCRm), situated at the Institut Pasteur of Côte d'Ivoire (IPCI), spanning from January 2012 to December 2020. The NCRm is the recipient of cerebrospinal fluid (CSF) samples from suspected cases of bacterial meningitis, sourced from all health districts across Côte d'Ivoire. The NCRm is responsible for diagnosing and confirming suspected cases of meningitis. The study included all CSF samples received at the NCRm between 2012 and 2020, encompassing both adults and children, provided they were accompanied by a properly completed epidemiological form or an analysis report. CSFs lacking an analysis sheet or bulletin were excluded from the study. Ethical approval was obtained from the appropriate authorities before the data analysis process commenced.

Epidemiological and laboratory data collection

All epidemiological data were collected from the epidemiological sheets accompanying the samples. Data analysis was performed using Microsoft Excel 2007. The variables used for the study were laboratory number, month of sample collection and year, patient age, sex, microscopy, culture, latex test, and polymerase chain reaction (PCR).

Cytobacteriological examination

The analysis was conducted employing conventional bacteriological methods, including macroscopy, microscopy (both in the fresh state and using Gram stain), cultures on blood-enriched agar media, bacterial identification based on biochemical characteristics, and the detection of soluble antigens (utilizing the Pastorex meningitidis kit from Biorad®, Marnes-la-Coquette, France).

Additionally, all CSF samples underwent molecular analysis through real-time PCR. The molecular study involved DNA extraction using the heat shock method, preparation of reaction mixtures, amplification, and gene detection. The targeted genes included Lyt A (*S. pneumoniae*), Hpd (*H. influenzae*), sodC C, and ctrA (*N. meningitidis*).

For *N. meningitidis* serotyping, the following targets were tested: csaB (*N. meningitidis A*), csb (*N. meningitidis B*), csc (*N. meningitidis C*), csy *(N. meningitidis Y*), csw (*N. meningitidis W*), and csxB (*N. meningitidis X*) (WHO, 2011).

RESULTS

For epidemiological characteristics, a total of 5046 CSF samples were analyzed at the National Center of Reference for Meningitis (CNRm) at the Institut Pasteur de Côte d'Ivoire. The mean age of the patients was 12 years, ranging from 0 to 86 years, with over 80% of the population falling between 0 and 5 years of age. The male/female sex ratio was 1.45, comprising 2987 males to 2059 females. Confirmed cases accounted for 476 or 8.3% of the total. The average age of confirmed patients was 10.2 years, with extremes ranging from 0 to 56 years, and the male/female sex ratio was 1.76. For the microbiological characteristics of CSF, Figure 1 illustrates the proportions of positivity by culture and PCR from 2012 to 2020.

The PCR technic detected the majority of positive meningitis cases from 2012 to 2020. In 2015, PCR-positive cases accounted for the majority, with 72%, compared with 11.59% in 2017. The culture-positive rate was low until 2020.From 5.4% in 2014 to 0 in 2020. Figure 2 represents the distribution of germs identified in CSF.

From 2012 to 2013, *N. meningitidis* was the most frequent pathogen, accounting for 59% in 2012 and 64% in 2013. Subsequently, from 2014 to 2020, *S. pneumoniae* became the most common germ, with rates

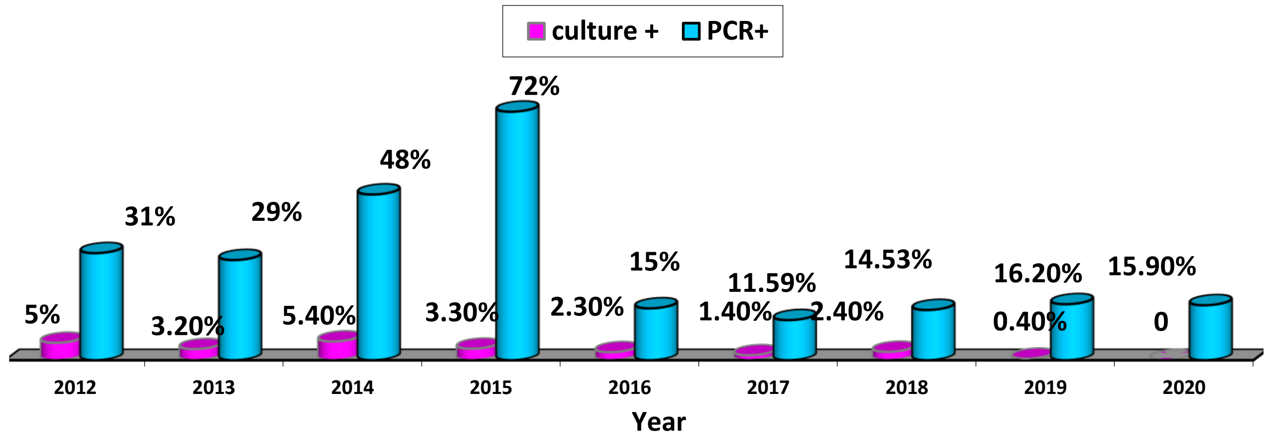


Figure 1. Proportions of positivity by culture and PCR from 2012-2020.

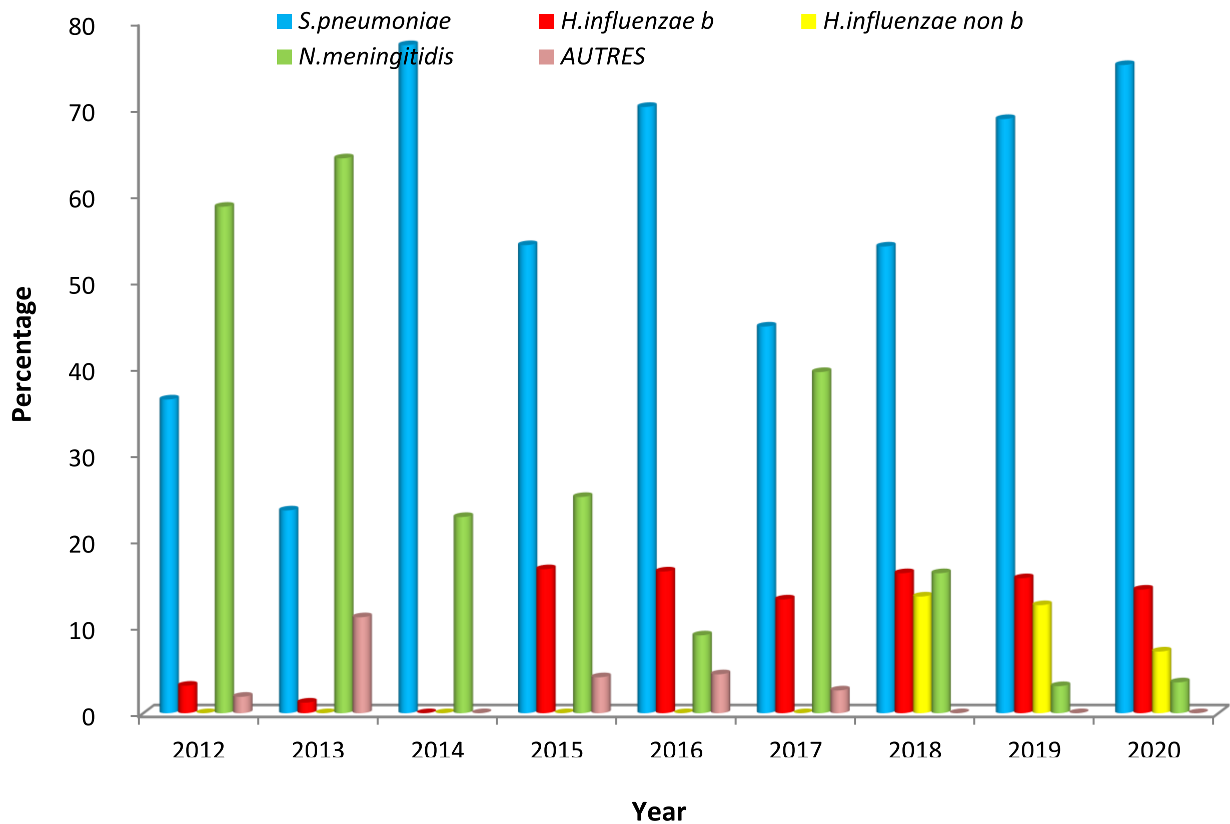


Figure 2. Distribution of germs identified in CSF from 2012-2020.
(Others): *S.pneumoniae H.influenzae b H.influenzae non b N.meningitidis*

ranging from 44.74 to 77.30%, followed by *N. meningitidis*. An emergence of *H. influenzae* b meningitis cases occurred from 2015 to 2020, with respective rates of 16.66 and 14.3%. Additionally, there was an emergence of cases of *H. influenzae* non-b meningitis, observed with rates ranging from 10 to 15% from 2018 to 2020. Other pathogens, such as *S. agalactiae*, *Escherichia coli* and *Salmonella* species, were found from 2012 to 2017, with rates ranging from 0 to 4%. Figure 3 represents the distribution of *N. meningitidis* by serogroup.

In 2012, *N. meningitidis* serogroup W135 was the

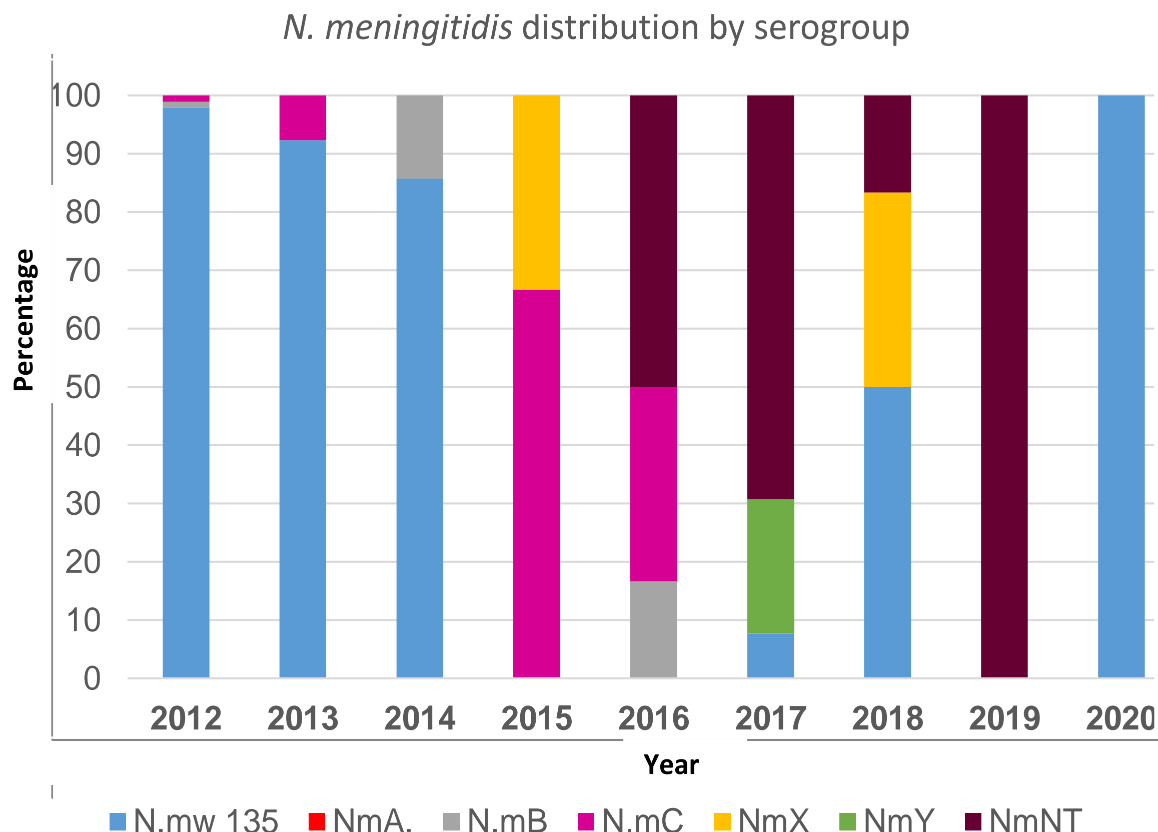


Figure 3. *N. meningitidis* distribution by serogroup from 2012-2020.

predominant pathogen until 2014, and then it appeared sporadically in other years, notably in 2018. No cases of *N. meningitidis* serogroup A were detected. There was an emergence of serogroup X in 2015 and 2017, and serogroup C persisted from 2012 to 2017. Serogroup X was reported in 2015 and 2018, while non-typeable *N. meningitidis* was observed from 2016 to 2020.

DISCUSSION

This study observed that more than 80% of the population with suspected meningitis cases were between 0 and 5 years of age, a result in agreement with Berthe (2020) findings in Mali. The male/female sex ratio was 1.45, closely aligned with the results of Savadogo et al. (2014) in Burkina Faso. Meningitis epidemics in African countries within the "meningitis belt" were predominantly caused by group *N. meningitidis* A (Lapeyssonnie, 1963). In 2012, *N. meningitidis* serogroup W135 was the predominant strain, comprising over 90% of cases. This could likely be attributed to the circulation of the same epidemiogenic clone responsible for meningitis epidemics in Senegal, Burkina Faso, Côte d'Ivoire, and 16 other belt countries in 2012 (Mustapha and Lee, 2018; WHO, 2013). Additionally, the observed shift may be attributed to the introduction of the meningococcal conjugate vaccine A, which may not provide protection against this serogroup (Mazamay et al., 2021; Mariagrazia et al., 2020). Subsequently, there was a progressive decline in *N. meningitidis* W from 2013 to 2019, decreasing from 93 to 0%, aligning with the findings of Ouangraoua in Burkina Faso in 2014 (Ouangraoua et al., 2014). However, serogroups X and Y emerged in 2015 and 2017, respectively. Hlozek et al., (2018) revealed that the similarity in the primary structures of the two polysaccharides suggests that serogroup A vaccination may offer cross-protection against serogroup X disease, yet some outbreaks caused by serogroup X have been reported. It should be noted, however, that non-typable serogroups existed from 2016 to 2019. Indeed, according to the World Health Organization (WHO), the introduction of the meningococcal conjugate vaccine A (MenAfriVac) strengthens the strategy for combating epidemics by improving prevention, but it also presents new challenges (WHO, 2013). Doumbia in Mali further revealed that after the introduction of MenAfriVac, *N. meningitidis* W became predominant, accounting for 66.10%, followed by pneumococcus at 22.88%, and *H. influenzae* b at 4.24%

(Doumbia, 2013). The global study by Pardo de Santayana, in summary, indicated that changes in invasive meningococcal disease over time were likely influenced by natural fluctuations, the emergence of virulent meningococcal clones (such as W:cc11), social trends, and immunization programs (Pardo de Santayana et al., 2023).

Furthermore, the rate of *S. pneumoniae*, which in 2012 was less than *N. meningitidis* W at 36%, underwent an increase with a frequency ranging from 23 to 70.15% from 2013 to 2016. These results align with the findings of Boni-Cisse et al. (2019) in 2019. From 2017 to 2020, it predominated over other pathogens with a rate ranging from 44.74 to 75%.

This shift is likely attributed to the introduction of the PCV10 conjugate vaccine in the Expanded Vaccine Program (Nhantumbo et al., 2015). Indeed, Kellner, (2011) noted that the marked decline in conjugate vaccine serotype infections was offset, to some extent, by an increase in infections attributable to a limited number of non-vaccine serotypes. Similarly, there was an increase in cases of *H. influenzae* b meningitis observed from 2015 to 2016. This surge in cases could be explained by the fact that the EPI only covers children aged 0 to 11 months. Booster doses are not being administered correctly, possibly due to the cost of vaccines and challenges in adhering to the vaccination calendar. Despite the availability of vaccines to protect against these serogroups, existing immunization programs do not cover all affected age groups (Clark, 2020). It is noteworthy that Slack (2021) in Australia affirmed that the Hib (*H. influenzae* b) conjugate vaccine has been successful in reducing the incidence of meningitis due to this germ to a very low level in countries where vaccination is well established in the EPI. However, much remains to be done in the case of non-immunized or partially immunized children. On the other hand, the emergence of non-b serotypes has become an important cause of meningitis in our populations, and these results are similar to those found among North American natives (Sadeghi-Aval et al., 2013; Ashley et al., 2019; Slack et al., 2021).

Conclusion

Bacterial meningitis constitutes a significant source of mortality and morbidity in Côte d'Ivoire. Vaccination against *N. meningitidis* has substantially altered the epidemiology of bacterial meningitis in high-risk regions. Meningitis-causing bacteria, such as *S. pneumoniae* and *H. influenzae*, have emerged. These bacteria, once regarded as non-epidemiogenic, were the primary isolates following the 2016 epidemic. Comprehensive epidemiological and microbiological surveillance, encompassing all age groups and types, is imperative to thoroughly document these changes and tailor vaccine regimens accordingly.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ashley C, Sarah B, Raymond SWT, Frances BJ, Julianne V , Marina U (2019). Continuing surveillance of invasive *Haemophilus influenzae* disease in northwestern Ontario emphasizes the importance of serotype a and non- typeable strains as causes of serious disease: a Canadian Immunization Research Network (CIRN). Canadian Journal of Microbiology 65(11):805-813. http://dx.doi 10.1139/cjm-2019-0210

Berthe IM (2020). Clinical bacteriological and therapeutic epidemiological study of bacterial meningitis in children aged 0 to 15 years in the Csref Msf of Koutiala. Thèses d'exercice de médecine générale. https://www.bibliosante.ml/handle/123456789/4137

Boni-Cisse C, Sheikh J, Rowan EB, Nicaise AL, Hamidou K, N'zue K, Alice BM, Flore SZ, Effua U, Peter S, Ndow AW, Jason MM, Joseph NB, Bernard N, Brenda AKA, Martin A (2019). Etiology of bacterial meningitis among children <5 years Old in Côte d'Ivoire : findings of hospital-based surveillance before and after pneumococcal conjugate vaccine introduction. Clinical Infectious Diseases 69(S2):114-20. http://dx doi 10.1093/cid/ciz475

Clark SA, Borrow R (2020). Herd Protection against Meningococcal Disease through Vaccination. Microorganisms. 8(11):675. http://dx doi: 10.3390/microorganisms8111675

Diallo A, Soeters HM, Yameogo I, Sawadogo G, Ake AF, Lingani C, Xin W, Andre B, Amadou F, Lassana S, Rasmata OT, Isaïe M, Brice B, Ryan TN (2017). MenAfriNet Consortium bacterial meningitis epidemiology and return of *Neisseria meningitidis* serogroup A cases in Burkina Faso in the five years following MenAfriVac mass vaccination campaign. PLoS ONE 12(11). http://dxdoi: 10.1371/journal.pone.0187466

Doumbia S (2013). Epidemiological and bacteriological aspects of meningitis in the district of Bamako before and after the introduction of Menafrivac: comparative study of data from the periods (2009-2010) and (2011-2012). Theses d'exercice de médecine générale. https://www.bibliosante.ml/handle/123456789/1734

Gervaix A (2014). Aseptic meningitis. Available at https://www.doc-developpement-durable.org/file/sante-hygiene-medecine/Maladies/meningite-aseptique/Meningite-aseptique_Hopital-Universitaire-de-Geneve.pdf

Hlozek J, Kuttel M, Ravenscroft N, Michelle J (2018). Conformations of *Neisseria meningitidis* serogroup A and X polysaccharides: The effects of chain length and O-acetylation. Carbohydrate Research Journal 30:465:44-51. http://doi: 10.1016/j.carres.2018.06.007

Kellner JD (2011). Update on the success of the pneumococcal conjugate vaccine Paediatrics and Child Health16(4):233-236. http://dx doi: 10.1093/pch/16.4.233

Kyaw MH, Christie P, Jones IG, Campbell H (2002). The changing epidemiology of bacterial meningitis and invasive non meningitis bacterial disease in Scotland during the period 1983-99. Scandinavian Journal of Infectious Diseases 34:289-298. http://doi: 10.1080/00365540110080403

Lapeyssonnie (1963). Cerebrospinal meningitis in Africa. Bulletin of the World Health Organization 28(Suppl):3-5 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2554630/

Mamadou KH , Dam K , Boubou L , Catherine O , Abdoulaye Y, Moussa H , Archibald W , Peter SN , Ricardo NO , Jason MM , Joseph B,Bernard N, Brenda A KA , Martin A (2019). Pediatric Bacterial Meningitis Surveillance in Niger Increased Importance of *Neisseria meningitidis* Serogroup C, and a Decrease in *Streptococcus pneumoniae* Following 13-Valent Pneumococcal Conjugate Vaccine Introduction. Clinical Infectious Diseases 5:69(Suppl 2):S133-S139 http://doi: 10.1093/cid/ciz598.

Mazamay S, Guégan, JF, Diallo, N, Bokabo E, Muyembe JJ, Taty N,

Vita TP, Broutin H (2021). An overview of bacterial meningitis epidemics in Africa from 1928 to 2018 with a focus on epidemics “outside-the-belt”. BMC Infectious Diseases 21:1027 https://doi.org/10.1186/s12879-021-06724-1

Mariagrazia P, Rafik BB, Rino R (2020). Vaccines against meningococcal Diseases. Microorganisms 3;8(10):1521. http://doi : 10.3390/microorganisms8101521

Medical Microbiology Course (2023). http://www.microbes-edu.org/

Mustapha MM, Lee HH (2018). Vaccine prevention of meningococcal disease in Africa major advances, remaining challenges. Human Vaccinnes Immunotherapeutics 14(5):1107-1115. http://dx. doi: 10.1080/21645515.2017.1412020

Nhantumbo AA, Cantarelli VV, Caireão J, Munguambe AM, Comé CE, Pinto GC, Zimba TF, Mandomando I, Semá CB, Dias C, Moraes MO, Gudo ES (2015). Frequency of pathogenic paediatric bacterial meningitis in Mozambique. The critical role of Multiplex Real Time Polymerase Chain Reaction to Estimate the Burden of Disease. PLoSOne 10(9):e0138249. http:// doi: 10.1371/journal.pone.0138249. eCollection 2015.

Ouangraoua S, Schlumberger M, Yaro S, Ouédraogo AS, Sanou S, Drabo A, Yaméogo TM, Ouedraogo R (2014). Impact of a meningococcal “A” conjugate vaccine on bacterial meningitis reported in western Burkina Faso (2009-2012). Bulletin of the Society of Exotic Pathology 107:27-30 https://doi.org/10.1007/s13149-014-0329-2

Pardo de Santayana C, Tin Tin Htar M, Findlow J, Balmer P (2023). Epidemiology of invasive meningococcal disease worldwide from 2010-2019: a literature review. Epidemiology and Infectious 6:151:e57. https://doi: 10.1017/S0950268823000328.

Sadeghi-Aval P, Tsang RSW, Frances B, Marina U (2013). Emergence of non-serotype b encapsulated *Haemophilus influenzae* as a cause of pediatric meningitis in northwestern Ontario. Canadian Journal of Infectious Disease and Medical Microbiology Spring 24(1):13-16 https:// doi: 10.1155/2013/828730

Savadogo M, Kyélem N, Yélbéogo D, Koussoubé F, Tarbagdo AO (2014). L’épidémie de meningitis à *Neisseria meningitidis* W135 en 2012 au Burkina Faso. Bulletin de la Société de Pathologie Exotique 107:15-17 https://doi.org/10.1007/s13149-013-0320-y

Sidikou F, Potts CC, Maman Zaneidou M,Sarah Mbaeyi S, Kadadé G, Paye MF, Ousmane S, Issaka B,Chen A, Chang HY, Issifou D, Lingani C,Sakande S, Bienvenu B, Mahamane AE, Diallo AO, Moussa A, Seidou I, Abdou M, Sidiki A, Garba O, Haladou S, Testa S, Nse RO, Mainassara HB, Wang X (2019). Epidemiology of bacterial meningitis in the nine years since Meningococcal Serogroup A Conjugate Vaccine Introduction, Niger, 2010-2018. Journal Infectious Diseases 31 :220(220 Suppl 4):S206-S215. https://doi: 10.1093/infdis/jiz296

Simonetta V (2022). Efficacy and effectiveness of the meningococcal conjugate Group A Vaccine MenAfriVac® in preventing recurrent meningitis epidemics in Sub-Saharan Africa. Vaccines 14 :10(4):617. doi: 10.3390/vaccines10040617

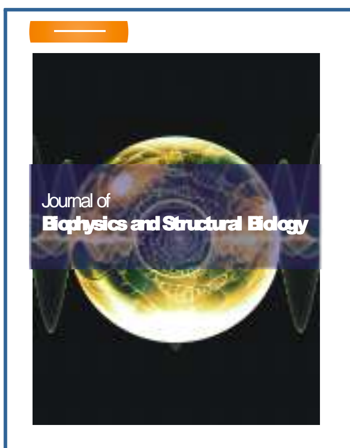
Slack MPE (2021). Long Term Impact of Conjugate Vaccines on *Haemophilus influenzae* Meningitis: Narrative Review. Microorganisms 9(5):886. https:// doi: 10.3390/microorganisms9050886

Slack MPE, Cripps AW, Grimwood K, Mackenzie GA, Ulanova M (2021). Invasive *Haemophilus influenzae* Infections after 3 Decades of Hib Protein Conjugate Vaccine Use. Clinical Microbiology Reviews 16(3):43. https://doi: 10.1128/CMR.00028-21. Epub 2021 Jun 2

World Health Organization and Centers for Disease Control and Prevention (U.S.) (2011). Laboratory methods for the diagnosis of meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae,* and *haemophilus Influenzae:* WHO Manual, 2nd Edition. https://iris.who.int/bitstream/handle/10665/70765/WHO_IVB_11.09_eng.pdf?sequenc

World Health Organization Geneva (2013). Meningococcal disease in countries of the African meningitis belt, 2012 emerging needs and future perspectives. Weekly Epidemiological Record 88(12):129-136 https://apps.who.int/iris/handle/10665/242046

Related Journals:



www.academicjournals.org