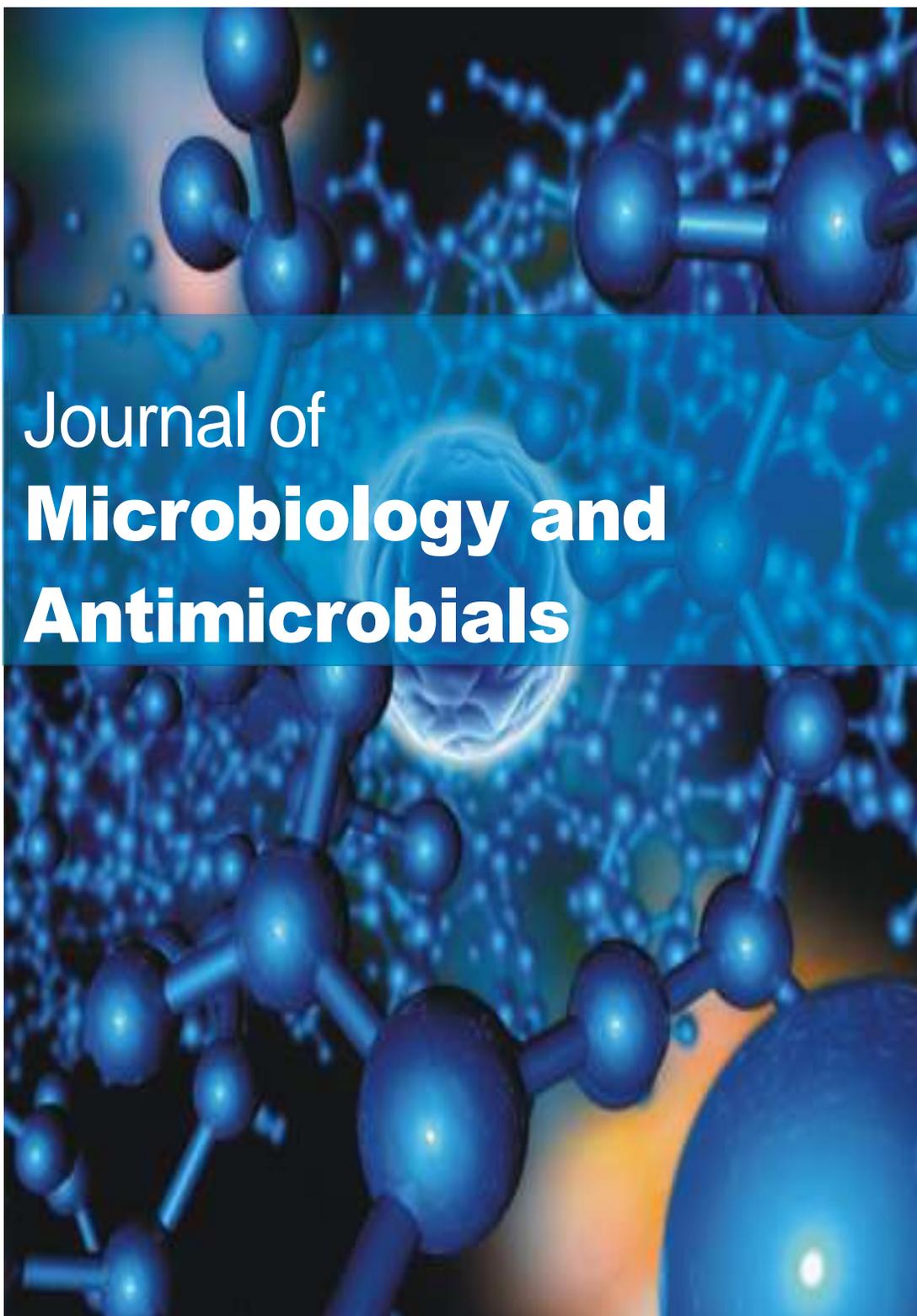


OPEN ACCESS



Journal of
**Microbiology and
Antimicrobials**

ABOUT JMA

The Journal of Microbiology and Antimicrobials (JMA) (ISSN 2141-2308) is published monthly (one volume per year) by Academic Journals.

Journal of Microbiology and Antimicrobials (JMA), is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as Disorders of the immune system, vaccines and antimicrobial drugs, Microbial Metabolism, Protozoology etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMA are peer-reviewed.

Contact Us

Editorial Office: jma@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: <http://academicjournals.org/JMA>

Submit manuscript online <http://ms.academicjournals.me/>

Editors

Ass. Prof. Aamer Ikram

*Department of Microbiology,
Armed Forces Institute of Pathology,
Pakistan*

Prof. Wang Jianhua

*Gene Engineering Lab
Feed Research Institute,
Chinese Academy of Agricultural Sciences
China*

Dr. Mohd. Shahid

*Antimicrobial Agents & Drug Section
Department of Medical Microbiology
Jawaharlal Nehru Medical College & Hospital
Aligarh Muslim University
India*

Dr. Anil Vyas

*Microbial Biotechnology & Biofertilizer Lab.
Department of Botany
J.N.V. University
India*

Dr. (Mrs.) Amita Jain

*Dept. of Microbiology
King George Medical University,
India*

Dr. Eduardo Mere

*Department of Biochemistry
University Federal of Rio de Janeiro,
Brazil*

Dr. Shwikar Mahmoud Abdel Salam

*Department of microbiology
Faculty of Medicine
Alexandria University
Egypt.*

Dr. Gideon Mutie Kikui

*Institute of Tropical Medicine and Infectious Diseases
Jomo Kentatta University of Agriculture and Technology
Kenya.*

Editorial Board Members

Dr. Manal El Said El Sayed

*Bilharz Research Institute (TBRI)
Ministry of Scientific Research
Egypt.*

Dr. Amber Farooqui

*Sardinian Research and Development (SARD)
Porto Conte Research Institute
Alghero,
Italy.*

Dr. Chang-Gu Hyun

*Laboratory of Bioresources
Jeju Biodiversity Research Institute (JBRI)
Jeju Hi-Tech Industry Development Institute (HiDI)
Korea.*

Dr. Vasant P. Baradkar

*Department of Microbiology
Government Medical College
Aurangabad,
India.*

Prof. Omar Abd El-Fattah Mohamed Fathalla

*Medicinal Chemistry Department
National Research Centre
Dokki,
Egypt.*

Dr. Amber Farooqui

*Dept. di Scienze Biomediche
Universita di Sassari
Italy.*

Dr. Kosta V. Kostov

*Military Medical Academy
Department of Pulmonology
Bulgaria.*

Dr. Antonio Rivera

*Benemérita Universidad Autónoma de Puebla
Puebla,
Mexico.*

Dr. Mohammad Rahbar

*Department of Microbiology
Iranian Reference Health Laboratory
Iran.*

Dr. Abd El-Latif Hesham

*Genetics Department
Faculty of Agriculture
Assiut University
Egypt.*

Dr. Samuel Sunday Taiwo

*Department of Medical Microbiology and Parasitology
College of Health Sciences
Nigeria.*

Dr. Anil Vyas

*J.N.V. University
Jodhpur
India.*

Dr. Najla Dar-Odeh

*University of Jordan
Jordan.*

Prof. Asiye Meric

*Anadolu University
Faculty of Pharmacy
Department of Pharmacy and Chemistry
Turkey.*

Prof. Salah M. Azwai

*AlFateh University
Libya.*

Prof. Abdel Salam Ahmed

*Department of Microbiology
Faculty of Medicine
Alexandria University
Egypt.*

Dr. Kuldeep Kumar Shivalya

*Indian Veterinary Research Institute
Izatnagar,
India.*

Prof. Viroj Wiwanitkit

*Hainan Medical University
China.*

Dr. Hafizah Chenia

*School of Biochemistry
University of KwaZulu-Natal
Durban,
South Africa.*

Dr. Gholamreza Salehi Jouzani

*Microbial Biotechnology and Biosafety Department
Agricultural Biotechnology Research Institute of Iran
(ABRII)
Iran.*

Dr. Wilson Parawira

*Institute of Food, Nutrition and Family Sciences
University of Zimbabwe
Zimbabwe.*

Dr. Subhash C. Mandal

*Division of Pharmacognosy
Department of Pharmaceutical Technology
Jadavpur University
India.*

Dr. Adesemoye A. O.

Department of Plant Pathology
Centre for Integrated Plant Systems
Michigan State University
USA.

Dr. Giselli Fernandes Asensi

Universidade Federal do Rio de Janeiro
Brazil.

Dr. Babu Joseph

Acharya's Bangalore School
India.

Dr. Aamer Ali Shah

Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad,
Pakistan.

Dr. Tadele Tolosa

Jimma University
College of Agriculture and Veterinary Medicine
Ethiopia.

Dr. Urveshkumar D. Patel

Department of Pharmacology and Toxicology
Veterinary College
Anand Agricultural University
India.

Dr. Saeed Zaker Bostanabad

Islamic Azad University
Iran.

Dr. Rakesh Kumar Singh

Florida State University
College of Medicine
USA.

Assoc. Prof. Vintila Iuliana

Dunarea de Jos University
Romania.

Dr. Saganuwan Alhaji Saganuwan

University of Agriculture Makurdi
Dept. of Physiology, Pharmacology and Biochemistry
Makurdi,
Nigeria.

Dr. Eskild Petersen

Dept. of Infectious Diseases
Aarhus University Hospital
Norbrogade,
Denmark.

Dr. Elpis Giantsou

Cambridge University Hospitals
UK.

Ass Prof. Emanu Getu Degaga

Addis Ababa University
Ethiopia.

Dr. Subramanian Kaviarasan

Dept of Molecular Medicine
University Malaya
Kuala Lumpur,
Malaysia.

Ass Prof. Nongyao Kasatpibal

Faculty of Nursing,
Chiang Mai University
Thailand.

Dr. Praveen Rishi

Panjab University
India.

Prof. Zeinab Nabil Ahmed Said

Microbiology & Immunology Department
Faculty of Medicine
Al-Azhar University
Egypt.

Ass. Prof. Abdulaziz Zorgani

Medical School
Edinburgh University
Edinburgh,
UK.

Dr. Adenike Adedayo Ogunshe

University of Ibadan
Nigeria.

Prof. Itzhak Brook

Department of Pediatrics and Medicine
Georgetown University
Washington, DC
USA.

Dr. Eduardo Mere Del Aguila

Universidade Federal do Rio de Janeiro
Rio de Janeiro
Brazil.

Dr. Md. Shah Alam Sarker

School Agric and Rural Development
Bangladesh Open University
Bangladesh.

Dr. Ramnik Singh

Khalsa College of Pharmacy
Amritsar,
India.

Prof. Amita Jain

Chhatrapati Shahuji Maharaj (CSM) Medical University
Lucknow,
India.

Prof. Yulong Yin

*Institute of Subtropical Agriculture
The Chinese Academy of Science
China.*

Prof. Mohan Karuppaiyl

*School of Life Sciences
Swami Ramanand Teerth Marathwada (SRTM) University
Maharashtra,
India.*

Dr. Sunil Gupta

*National Centre for Disease Control
India.*

Dr. Elpis Giantsou

*Cambridge University Hospitals
England.*

Dr. Mustafa Gul

*Kahramanmaras Sutcuimam University
Faculty of Medicine
Department of Microbiology and Clinical Microbiology
Turkey.*

Dr. Nese Karaaslan Biyikli

*Anadolu Medical Center
Turkey.*

Dr. Zafar Iqbal

*Dept Plant Pathology
University College of Agriculture
András Fodor
Pakistan.*

Ass Prof. Habil András Fodor

*Department of Plant Protection
Georgikon Faculty
Pannonia University
Hungary.*

Dr. Neelam Mewari

*Department of Botany
University of Rajasthan
Rajasthan,
India.*

Dr. Elpis Giantsou

*Cambridge University Hospitals
UK.*

Dr. Sanjib Bhattacharya

*Bengal School of Technology
India.*

Dr. Habibur Rahman

*PSG Colege of Pharmacy
India.*

Md. Elisa Bassi

*Department of Dermatology
Delmati Hospital
Italy.*

Iheanyi Omezuruike Okonko

*University of Ibadan
Nigeria.*

Ass. Prof. Weihua Chu

*Dept. of Microbiology
School of Life Science & Technology
China Pharmaceutical University
China.*

Dr. Mat Yamage

*World Organization for Animal Health (OIE)
Japan.*

Dr. Ali Abbas Qazilbash

*United Nations Industrial Development Organization
Pakistan.*

Dr. Kulachart Jangpatarapongsa

*Department of Clinical Microbiology
Mahidol University
Thailand.*

Dr. Nasrin Ghasemi

*Research and Clinical Centre for Infertility
Yazd Shahid Sadoughi University of Medical Sciences
Yazd,
Iran.*

Dr. Johnson Afonne

*Department of Pharmacology
College of Health Sciences
Nnamdi Azikiwe University
Nigeria.*

Dr. Branka Vasiljevic

*Institute of Molecular Genetics and Genetic Engineering
Serbia.*

Dr. Mehmet Ulug

*BSK Anadolu Hospital
Infectious Diseases and Clinic Microbiology
Turkey.*

Dr Ömür Baysal

*Turkish Ministry of Agriculture and Rural Affairs
West Mediterranean Agricultural Research Institute
(BATEM)
Plant Pathology and Molecular Biology Departments
Antalya,
Turkey.*

Dr. Pooja Jain

*University of California
Department of Pathology
Medical Sciences
Irvine, CA
USA.*

Dr. Chellaiah Edward Raja

*Cancer Biology Unit
School of Biological Sciences
M.K. University
India.*

Prof. Zeinab Nabil Ahmed Said

*Faculty of Medicine (for girls)
Al-Azhar University
Egypt.*

Prof. Manal Mohammad Baddour

*Alexandria University
Faculty of Medicine
Microbiology and Immunology Dept.
Azarita,
Egypt.*

Dr. Bechan Sharma

*Department of Biochemistry
Centre for Biotechnology
University of Allahabad
Allahabad,
India.*

Ass. Prof. Ravichandran Veerasamy

*Faculty of Pharmacy
AIMST University
Malaysia*

Dr. Mohammad Ibrahim

*Programa de Pós-Graduação em Bioquímica Toxicológica
Centro de Ciências Naturais e Exatas
Universidade Federal de Santa Maria
Brazil.*

Dr. Sudheer Bobba

*Department of Drug Metabolism and Pharmacokinetics
Covance Laboratories
USA.*

Dr. Kannan Alpadi

*Department of Molecular Biology and Biochemistry
Baylor College of Medicine
USA.*

Dr. Shaohua Chen

*Department of Plant Pathology
South China Agricultural University
Guangzhou,
China.*

Dr. Prasun Kumar

*Department of Microbial Biotechnology and Genomics
CSIR-Institute of Genomics and Integrative Biology
India.*

Table of Content

Assessment of microbiological quality of kebab sold on the campus of a tertiary education and its environs in Ghana Azumah Ayamah ¹ , Augustina Angelina Sylverken and Linda Aurelia Andoh	20
Microbiological profile of asymptomatic bacteriuria in pregnant women in Volta Region, Ghana Dzifa Kofi Ahiatrogah, Jude John Annan, Linda Aurelia Ofori, Benedict Awudzi, Alex Owusu-Ofori, Michael Owusu, and Augustina Angelina Sylverken	27
Goat carcass microbial investigation in Modjo Export Abattoirs, Ethiopia Abraha Negash and Karanfil Olga	37

Full Length Research Paper

Assessment of microbiological quality of kebab sold on the campus of a tertiary education and its environs in Ghana

Azumah Ayamah^{1,2*}, Augustina Angelina Sylverken² and Linda Aurelia Andoh²

¹Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

²Department of Theoretical and Applied Biology, Faculty of Biosciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Received 18 March, 2021; Accepted 3 June, 2021

Prevalence and incidence of foodborne illness in developing countries has risen in recent times as a result of increased demand for Ready-To-Eat (RTE) foods. The current study assessed the microbiological quality of kebab sold at selected areas within the Kumasi metropolis. A total of 36 kebab samples were purchased and analyzed for Total Viable Count (TVC), Total Coliforms Count (TCC) and Thermotolerant Coliforms Count (TTC). The results showed that the mean TVC, TCC and TTC in beef kebab at the different locations ranged from 6.91- 7.23 Log₁₀ CFU/g, 7.25-9.23 Log₁₀ MPN/g and 4.97-7.75 Log₁₀ MPN/g respectively. For chevon kebab, it ranged from 6.83-7.25 Log₁₀ CFU/g, 7.98 - 9.23 Log₁₀ MPN/g and 6.61-8.81 Log₁₀ MPN/g respectively. That of gizzard kebab ranged from 6.89-7.30 Log₁₀ CFU/g, 7.98-9.23 Log₁₀ MPN/g and 6.89-7.53 Log₁₀ MPN/g respectively. The mean TVC, TCC and TTC for the beef kebab were not significant ($p = 0.680, 0.055$ and 0.070) respectively. For the chevon, the TVC and TCC were not significant ($p = 0.547$ and 0.121) respectively but that of the TTC was significant ($p = 0.034$). The mean TVC, TCC and TTC of the gizzard were not significant ($p = 0.794, 0.056$ and 0.822) respectively at the different locations. These mean microbial loads (TVC, TCC and TTC) in the kebab samples exceeded the standard acceptable limits ($< 5 \text{ Log CFU/g}$ and $< 2 \text{ Log}_{10} \text{ MPN/g}$). Since the microbial loads exceeded the standard acceptable limits, it could put consumers at high risk of contracting foodborne infection. This result should prompt the relevant institutions responsible for ensuring food safety in the metropolis to strictly enforce the standard regulations on food safety practices as well as carry out adequate monitoring to avoid possible foodborne infections.

Key words: Kebabs, total viable count, total coliforms count, thermotolerant coliforms count.

INTRODUCTION

Street food vending plays an important role in the financial growth of people living in the cities of Ghana and other developing nations (Osei Mensah et al., 2016). In

addition, the economy of a nation gets bolstered through the taxes paid by these vendors (Okoye, 2020; Anyidoho, 2013). The industry provides large collection of foods that

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

are relatively cheap, nutritious and easy to come by to thousands of consumers every day (Tigari and Shalini, 2020; Bellia et al., 2016; Monney et al., 2014; Tambekar et al., 2008).

Despite its important role, foodborne infections of microbial source are the main health risks associated with its consumption (Gizaw, 2019; Cho et al., 2011; El-Shenawy et al., 2011; Campos et al., 2015; Mensah et al., 2002). The total number of outpatients reported cases of foodborne infections in Ghana is about 420,000 per year, with an annual death rate estimated at 65,000 and total cost to the economy at \$69 million (Graphic Online, 2015; Mahami and Odonkor, 2012; MOFA and World Bank, 2007). The problem of foodborne infection in developing countries is mainly due to failure of the street food vendors to comply with the standard guidelines during preparation of the food and secondary contamination after preparation (Feglo and Sakyi, 2012; Tavakoli, 2008). Additionally, lack of food safety training for vendors have equally contributed to the causes of these foodborne infections since majority of them operate in poor hygienic conditions (Umar et al., 2018). Estrada-Garcia et al. (2004) indicated that street foods are the commonest source of foodborne disease outbreaks. It is worth noting that food safety issues are not restricted to only developing countries. A significant number of food related outbreaks have been reported in most developed countries. In the selected countries in USA and Europe, rates of death are as high as 3000 and 4654 annually (Scallan et al., 2011a, b; WHO, 2017).

Khebab belongs to these Ready-To-Eat (RTE) foods. It is popularly known in Ghana as 'chinchinga' which is highly patronized. Among the RTE foods, there are indications that khebab may be the most patronized (Panozzo et al., 2015). As such, it is usually sold at the street corners, markets, beaches, lorry terminals, beer bars and restaurants. The khebab is usually made from either beef, pork, chevon, mutton, chicken or other sources of meat. The meat is marinated with a preparation called 'suya' spice and char-grilled. Suya is a spicy meat skewer which is a popular food item in West Africa. During the processing of the khebab, it is extensively manipulated by the vendor key among being the constant touching and turning of the meat whilst being grilled. Such activities have a potential for the introduction of high microbial contamination. In addition, some portions of the meat stuck on the stick and may not be well-cooked. As a result, there may be an increased risk of pathogen survival not only by cross-contamination, but also by undercooking. There have been several reported incidences of foodborne disease outbreaks associated with khebab consumption (ACMSF, 2004; Evans et al., 1999; Little and Gillespie, 2008). For instance, in England and Wales, several foodborne diseases outbreaks associated with khebabs have been reported by Meldrum et al. (2009). Additionally, Synnott et al. (1993) also reported an outbreak of *Salmonella*

mikawasima which was associated with doner khebabs consumption. Recent studies have indicated unsatisfactory loads of bacteria $> 10^5$ cfu/g in khebab samples (Durmaz et al., 2015; Lopašovský et al., 2016). At KNUST for instance, majority of students, staff and indigenous people residing on KNUST campus and its environs patronize these ready-to-eat foods (Ababio and Adi, 2012). The Ghana Standard Board has indicated an acceptable limit of <5 Log CFU/g for bacteria in khebab. As such, any khebab beyond this threshold when consumed may have dire public health implications.

Even though khebab is one of the most patronized of such foods, the microbial profile as well as load has not yet been established. Therefore, the present study estimated the load and further profile bacteria contaminants in khebab sold on the campus of a tertiary institution and its surrounding communities in Ghana.

MATERIALS AND METHODS

Study design

A cross-sectional study was conducted to assess the microbiological quality of ready-to-eat meat (khebab) sold in KNUST campus and its environs.

Study location

The study was conducted at the Kwame Nkrumah University of Science and Technology (KNUST) campus and selected areas including Ayigya, Kotei, Ayeduase, Boadi and Kentinkrono in the Ashanti region of Ghana (Figure 1).

Sample collection

RTE khebab made from beef, chevon and gizzard were purchased from each vendor in KNUST campus, Ayigya, Kotei, Ayeduase, Boadi and Kentinkrono. At each location, the khebabs were purchased from two vendors. About one hundred grams of the three khebab samples (beef, chevon and gizzard) were placed in labeled sterile ziploc bags. The samples were kept in an ice chest with ice packs and transported immediately to the laboratory for analysis.

Microbiological analysis

Total viable count (TVC)

The total viable count (TVC) was carried out using the pour plate technique with plate count agar (PCA) (Oxoid CM 0325; Oxoid Ltd Basingstoke, Hampshire, England). A stock dilution was prepared by placing 10 g of khebab sample into 90 ml sterilized buffered peptone water (Oxoid CM 0009; Oxoid Ltd Basingstoke, Hampshire, England) and pulsed for 15 s. The stock was serially diluted by transferring 1 ml of it into 9 ml sterile peptone water and successfully transferred in a similar manner to obtain dilution of 10^{-2} to 10^{-5} . Aliquots of 1 ml from each of the dilutions were put into labeled Petri dishes and about 10 ml of molten (45°C) plate count agar was added. The plates were swirled slowly for uniform mixing and allowed to solidify and sealed with parafilm and incubated at

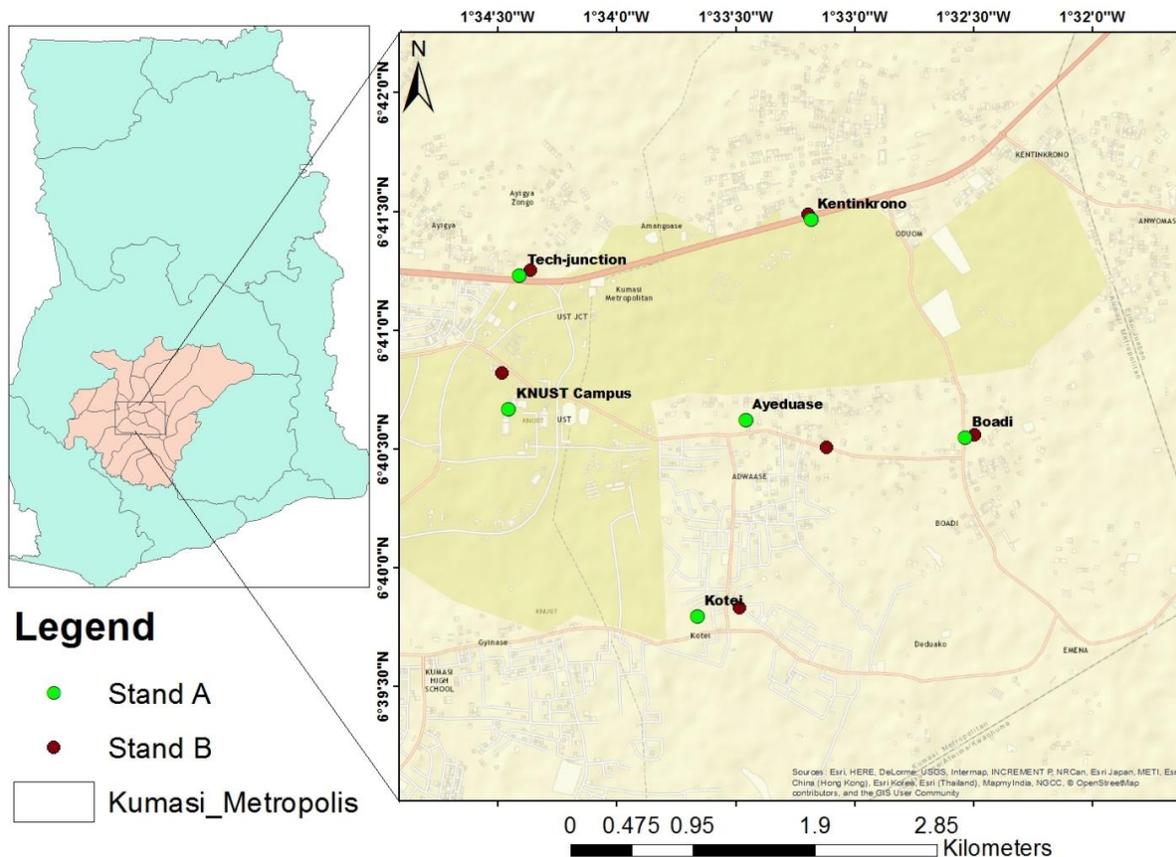


Figure 1. Map showing sampling locations.

36°C (± 1) for 24 h. After the incubation, colonies between 30 and 300 were counted using the colony counter (Stuart colony counter, UK) and the average colonies were calculated, from which the total viable count was estimated using the formula:

$$\text{Colony forming unit (cfu)} = \text{Average colonies} \times \frac{\text{Dilution factor}}{\text{Aliquot plated}}$$

and expressed as CFU/g.

Total and thermotolerant coliforms

The Most Probable Number (MPN) method was employed to determine the total and thermotolerant coliforms in the khebab samples. One milliliter aliquots from each of the dilutions prepared were inoculated into a 5 ml sterile MacConkey Broth (Oxoid CM 0085; Oxoid Ltd Basingstoke, Hampshire, England) and incubated at 36°C (± 1) for total coliforms and 44°C for Thermotolerant coliforms for 24 h. The tubes that showed colour change from purple to yellow after 24 h were identified as positive for both total and Thermotolerant coliforms. Counts per gram were calculated from the MPN tables. The total number of organisms in the samples was estimated using the formula:

$$\text{MPN/g} = \text{Number of organisms obtained from MPN table} \times \text{Dilution factor of middle set of tubes}$$

(<https://www.jlindquist.com/generalmicro/102dil3.html>).

Statistical analysis

The data obtained were analyzed by Genstat statistical software (version 12) (Payne et al., 2009). The means of bacterial load (TVC and MPN) of samples collected from the various sites were compared using One-way Analysis of Variance (ANOVA). Significant differences were assessed at 5% level of significance ($p = 0.05$). Where there was significant difference, means were separated using the Fishers protected least significant difference (LSD) procedure to identify the source of difference. The data were log arithmetically (Log_{10}) transformed to minimize the variations associated with the enumeration techniques.

RESULTS

Microbial load of beef khebab samples from the six locations

In general, the khebab samples analyzed showed varying loads of microbes. Table 1 shows that KNUST campus recorded the highest mean TVC (7.23 Log_{10} CFU/g), while Kotei recorded the least (6.91 Log_{10} CFU/g). The results showed no significant differences ($p = 0.680$) among the mean TVC loads (Table 1). For the total coliform count, Ayigya recorded the highest mean load

Table 1. Microbial load of beef kebab samples from KNUST campus and its environs.

Location	TVC range (CFU/g)	Mean TVC (Log ₁₀ CFU/g)	TCC range (MPN/g)	Mean TCC (Log ₁₀ MPN/g)	TTC range (MPN/g)	Mean TTC (Log ₁₀ MPN/g)
Kotei	4.00×10 ⁵ -1.92×10 ⁷	6.91 ± 0.54	2.10×10 ⁶ -2.40×10 ⁹	8.01 ± 1.38	9.00×10 ⁵ -2.40×10 ⁷	6.84 ± 0.68
Ayeduae	9.20×10 ⁴ -2.48×10 ⁷	6.97 ± 0.83	4.00×10 ⁶ -2.40×10 ⁹	8.15 ± 1.26	2.30×10 ⁵ -9.3×10 ⁷	6.88 ± 1.21
Kentinkrono	5.60×10 ⁶ - 2.44×10 ⁷	7.19 ± 0.24	1.50×10 ⁸ -9.30×10 ⁸	8.70 ± 0.41	2.30×10 ⁷ -7.50×10 ⁷	7.62 ± 0.23
Boadi	1.03×10 ⁶ -2.52×10 ⁷	7.06 ± 0.48	1.50×10 ⁵ -2.40×10 ⁹	7.25 ± 1.88	9.00×10 ⁶ -9.30×10 ⁷	4.97 ± 3.88
KNUST-campus	9.10×10 ⁶ - 2.68×10 ⁷	7.23 ± 0.18	2.10×10 ⁸ -2.40×10 ⁹	8.88 ± 0.47	2.30×10 ⁷ -9.30×10 ⁷	7.65 ± 0.27
Ayigya	8.10×10 ⁶ -2.32×10 ⁷	7.19 ± 0.15	9.00×10 ⁸ -2.40×10 ⁹	9.23 ± 0.21	1.50×10 ⁶ -4.30×10 ⁷	7.75 ± 0.34
CV (%)		6.6		13.3		24.4
<i>p-value</i>		0.680		0.055		0.070

TVC= Total Viable Count, TCC = Total Coliform Count, TTC= Thermotolerant Coliform Count, MPN= Most Probable Number, CFU= Colony Forming Unit, CV= Coefficient of variation.

Table 2. Microbial load of chevon kebab from KNUST campus and its environs.

Location	TVC range (CFU/g)	Mean TVC (Log ₁₀ CFU/g)	TCC range (MPN/g)	Mean TCC (Log ₁₀ MPN/g)	TTC range (MPN/g)	Mean TTC (Log ₁₀ MPN/g)
Kotei	8.20×10 ⁵ -2.18×10 ⁷	6.83 ± 0.60	2.30×10 ⁶ -9.30×10 ⁸	7.98 ±1.26	2.30×10 ⁵ -4.30×10 ⁷	6.61± 1.03 ^a
Ayeduae	3.50×10 ⁵ -2.80×10 ⁷	7.03 ± 0.64	9.00×10 ⁵ -2.40×10 ⁹	7.98 ± 1.60	2.30×10 ⁵ -9.30×10 ⁷	6.88 ± 1.21 ^a
Kentinkrono	3.90×10 ⁶ - 2.76×10 ⁷	7.22 ± 0.30	7.00×10 ⁷ -2.90×10 ⁸	8.23 ± 0.29	9.30×10 ⁶ -9.30×10 ⁷	7.53 ±0.47 ^{ab}
Boadi	4.70×10 ⁶ -2.60×10 ⁷	7.17 ± 0.27	2.10×10 ⁸ -2.40×10 ⁹	8.72 ±0.51	1.60×10 ⁷ -4.30×10 ⁷	7.38 ±0.19 ^{ab}
KNUST- Campus	9.20×10 ⁶ - 7.74×10 ⁷	7.25 ± 0.31	9.00×10 ⁸ -2.40×10 ⁹	9.23 ± 0.21	2.90×10 ⁷ -2.90×10 ⁹	8.18 ± 1.00 ^b
Ayigya	4.70×10 ⁵ -2.96×10 ⁷	7.07 ± 0.59	2.90×10 ⁸ -9.30×10 ⁸	8.68 ±0.23	1.50×10 ⁷ -9.30×10 ⁷	7.53 ±0.40 ^{ab}
CV (%)		6.70		10.3		11.0
<i>p-value</i>		0.547		0.121		0.034

TVC= Total Viable Count, TCC = Total Coliform Count, TTC= Thermotolerant Coliform Count, MPN= Most Probable Number, CFU= Colony Forming Unit, a, b = Mean within column with the same letter for same count are not significantly different ($p > 0.05$); CV= Coefficient of variation.

(9.23 Log₁₀ MPN/g) whereas Boadi recorded the least (5.25 Log₁₀ MPN/g). Again, KNUST campus recorded the highest thermotolerant coliform count (7.75 Log₁₀ MPN/g) while Boadi recorded the least (4.97 Log₁₀ MPN/g). There were no statistical differences ($p = 0.055$ and 0.070) among the mean loads for the total and thermotolerant coliform counts, respectively.

Microbial load of chevon kebab samples from the six locations

Khebab samples from KNUST campus recorded the highest mean TVC of 7.25 Log₁₀ CFU/g, whereas those from Kotei recorded the least mean TVC load of 6.83 Log₁₀ CFU/g (Table 2). The differences in the mean loads were not significant ($p = 0.55$). Likewise, khebab samples from KNUST campus recorded the highest mean total coliform load of 9.23 Log₁₀ MPN/g while those from Kotei and Ayeduae recorded the least (7.98 Log₁₀ MPN/g). Similarly, khebab samples on KNUST campus recorded the highest mean

total coliforms load of 8.18 Log₁₀ MPN/g while those of Kotei showed the least (6.61 Log₁₀ MPN/g). There were no significant differences ($p = 0.12$) among the mean loads of the total coliform count recorded. However, the mean load of thermotolerant coliforms recorded for those from KNUST campus was significantly higher than that of Kotei and Ayeduae ($p = 0.043$).

Microbial load of gizzard kebab samples from the six locations

For total viable count, khebab samples from Ayigya recorded the highest mean load of 7.30 Log₁₀ CFU/g while those from KNUST campus recorded the least (6.88 Log₁₀ CFU/g). Samples from Ayigya also recorded the highest mean load of total coliforms of 9.23 Log₁₀ MPN/g, while samples from Ayeduae recorded the least mean load of total coliforms (7.98 Log₁₀ MPN/g) (Table 3). Kentinkrono recorded the highest mean thermotolerant coliform load of 7.53 Log₁₀ MPN/g, while the lowest mean load of 6.89

Table 3. Microbial load of gizzard kebab from KNUST campus and its environs.

Location	TVC range (CFU/g)	Mean TVC (Log ₁₀ CFU/g)	TCC range (MPN/g)	Mean TCC (Log ₁₀ MPN/g)	TTC range (MPN/g)	Mean TTC (MPN/g)
Kotei	1.85×10 ⁶ -2.60×10 ⁷	7.12 ± 0.38	9.00×10 ⁶ -9.30×10 ⁸	8.13 ± 0.93	1.50×10 ⁶ -2.10×10 ⁷	6.89 ±0.56
Ayeduase	3.00×10 ⁴ -2.68×10 ⁷	6.90 ± 1.00	4.00×10 ⁵ -2.40×10 ⁹	7.98 ± 1.84	2.30×10 ⁶ -2.90×10 ⁹	7.43 ±1.83
Kentinkrono	6.50×10 ⁵ - 2.96×10 ⁷	6.89 ± 0.65	2.90×10 ⁸ -2.40×10 ⁹	9.07 ± 0.47	1.50×10 ⁷ -9.30×10 ⁷	7.53 ± 0.36
Boadi	8.20×10 ⁵ -2.64×10 ⁷	7.05 ± 0.52	4.00×10 ⁸ -2.40×10 ⁹	9.12 ± 0.39	2.10×10 ⁷ -2.90×10 ⁷	7.39 ± 0.064
KNUST-Campus	3.20×10 ⁴ - 2.84×10 ⁷	6.88 ± 0.99	9.00×10 ⁷ -2.90×10 ⁸	8.25 ± 0.23	4.00×10 ⁷ -9.30×10 ⁷	7.40 ± 0.62
Ayigya	1.24×10 ⁷ -2.80×10 ⁷	7.30 ± 0.147	9.00×10 ⁸ -2.40×10 ⁹	9.23 ± 0.21	2.10×10 ⁷ -2.10×10 ⁷	7.32 ± 0.00
CV (%)		9.8		10.3		11.4
<i>p-value</i>		0.794		0.056		0.822

TVC= Total Viable Count, TCC = Total Coliform Count, TTC= Thermotolerant Coliform Count, MPN= Most Probable Number, CFU= Colony Forming Unit.

Log₁₀ MPN/g was observed in Kotei for thermotolerant coliform count.

DISCUSSION

Though the mean TVC load obtained for the beef, chevon and gizzard kebab samples were different; they showed no significant differences ($p = 0.680, 0.545$ and 0.794), respectively at the different sampling locations. However, the TVC for the different kebabs in all the locations exceeded the Ghana Standard Board acceptable limit of <5 Log CFU/g. This could mean similar hygienic and processing methods were undertaken by the vendors at the different locations. Since the mean TVC of the kebabs were above the standard acceptable limit, they were unsatisfactory for human consumption (NSW Food Authority, 2009). It also suggests the existence of microbial risk associated with the different kebabs in the different locations. Our results on TVC can be compared to a similar study by Manguiat and Fang (2013) and Ologhobo et al. (2010) who reported unsatisfactory levels of aerobic plate count (APC) in hot grilled chicken and Nigerian roasted chicken 'suya', respectively. Shaltout et al. (2015) also reported unsatisfactory levels of aerobic plate count (APC) in street vended meat products sandwiches in Kalyobia Governorate. Further, the study agree with findings of Wimalasekara and Gunasena (2016) who reported unacceptable levels of TVC in RTE Kebab and Ham with Log₁₀CFU ranging (4.34-8.53) and (6.42-8.53), respectively.

The mean total coliform count (TCC) and thermotolerant coliform count (TTC) of beef, chevon and gizzard kebabs at the different locations were all above the standard acceptable limit of <2 Log MPN/g. Consumers are therefore at risk of contracting pathogenic bacteria infections such as typhoid fever (*Salmonella* species), dysentery (*Shigella* species), cholera (*Vibrio cholera*), etc. In Ghana, from 2009 to 2013, there were four main clinically diagnosed foodborne diseases which include

cholera (59.8%), typhoid fever (16.5%), dysentery (shigellosis) (2.6%) and viral hepatitis (1.6%) (Osei-Tutu and Anto, 2016). Although the mean TCC and TTC for kebab from different meats were different at each location, they were not significantly different ($p > 0.05$) except for the mean TTC of the chevon kebab which showed significant differences among KNUST campus, Kotei and Ayeduase ($p = 0.034$). Similarity in bacterial loads of samples collected at the various locations could be attributed to the fact that the kebab vendors were operating under similar environmental conditions. Similar study by Manguiat and Fang (2013) reported unsatisfactory levels of coliforms (4.4 Log CFU/g) in hot grilled chicken in Laguna, Taiwan. Agbodaze et al. (2005) also reported unsatisfactory levels of faecal coliforms in kebabs sampled from Accra central (4.4 Log CFU/g), Osu (3.98 Log CFU/g) and Nima (3.80 Log MPN/g) all in Ghana. Wimalasekara and Gunasena (2016) also reported unacceptable levels of total coliforms in RTE Kebab and Ham with Log₁₀ MPN ranging (0.95-3.04) and (2.66-3.04), respectively as well as unacceptable levels of faecal coliforms ranging (0.60-3.04) and (2.66-3.04), respectively.

The high mean TVC, TCC and TTC load observed in the beef, chevon and gizzard kebab could be as a result of poor storage conditions and poor handling processes by the vendor as suggested by NSW Food Authority (2009). According to Ababio and Adi (2012) and Fang et al. (2003), most RTE food vendors are not educated and lack the knowledge in hygienic practices and safety of the food product and this may lead to their contamination.

Factors such as educational level, knowledge in hygienic practices such as frequent washing of hands, protection of food from flies and dust, etc. have been reported as determinants of foodborne infections (Amegah et al., 2020; Monney et al., 2013; Yiğit and Duran, 1997). Elsewhere, there are indications that majority of kebab vendors do not keep the kebab in the display cabinet and therefore are exposed to the mercy of the environment and this could be a route through which

the kebab can be contaminated by polluted air and dust as well as flies which are in the environment. The metal bars used in roasting the kebabs if not properly cleaned could also introduce microorganisms into the meat and cause contamination. Schroeder et al. (2005) and Mbotto et al. (2012) indicated that the presence of high faecal coliforms in foods depicts poor hygienic practices of handling the meat during slaughtering and processing. Other factors such as cross contamination from the skin, mouth or nose of the handlers could also introduce pathogens into the meat. Contamination may also arise from the spices used by vendors as well as the heating process, if not done properly to kill or minimize the loads of microorganisms.

Conclusion

The study in general has demonstrated that street vended kebab sold on KNUST campus, Ayigya, Kotei, Ayeduase, Kentinkrono and Boadi were significantly contaminated with varying loads of microbes. Microbial levels were above the Ghana Standard Board and Brazilian food sanitation standard acceptable bacterial limits established for total viable count (TVC), total and thermotolerant coliform count. Thus, consumers are at a high risk of contracting infections and therefore food vendors should be given frequent training such as seminars, workshops and public education on the consequences of poor personal and sanitation hygiene. Additionally, the Municipal Health Inspectors must monitor the activities of these Ready-To-Eat food vendors to ensure strict adherence to the safety regulations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Ababio PF, Adi DD (2012). Evaluating food safety practices among food handlers in the Kumasi Metropolis. *Internet Journal of Food Safety* 14(2):35-43.
- Advisory Committee on the Microbiological Safety of Food (ACMSF) (2004). *Microbiological Control of Doner Kebabs*. Food Standards Agency, London.
- Agbodaze D, Nmai PN, Robertson F, Yeboah-Manu D, Owusu-Darko K, Addo K (2005). Microbiological quality of kebab consumed in the Accra metropolis. *Ghana Medical Journal* 39(2):46-49.
- Amegah KE, Addo HO, Ashinyo ME, Fiagbe L, Akpanya S, Akoriyea SK, Dubik SD (2020). Determinants of hand hygiene practice at critical times among food handlers in educational institutions of the Sagnarigu municipality of Ghana: a cross-sectional study. *Environmental Health Insights* 14:1178630220960418.
- Anyidoho NA (2013). *Informal Economy Monitoring Study: Street Vendors in Accra, Ghana*. Manchester, United Kingdom: Women in Informal Employment: Globalizing and Organizing.
- Bellia C, Pilato M, Seraphin H (2016). Street food and food safety: a driver for tourism? *Calitatea* 17(S1):20-27.
- Campos J, Gil J, Mourao J, Peixe L, Antunes P (2015). Ready-to-eat street-vended food as a potential vehicle of bacterial pathogens and antimicrobial resistance: an exploratory study in Porto region. *Portugal International Journal of Food Microbiology* 206:1-6.
- Cho JI, Cheung CY, Lee SM, Ko SI, Kim KH, Hwang IS, Kim SH, Cho SY, Lim CJ, Lee KH, Kim KS, Ha SD (2011). Assessment of microbial contamination levels of street-vended foods in Korea. *Journal of Food Safety* 31:41-47.
- Durmaz H, Aygun O, Sancak H (2015). The microbiological quality of grilled meats (Kebab) and salads consumed in Sanliurfa restaurants. *International Journal of Science and Technology Research* 1(1):297-302.
- El-Shenawy M, El-Shenawy M, Mañes J, Soriano JM (2011). *Listeria spp.* in street vended ready-to-eat foods. *Interdisciplinary Perspective on Infectious Disease* <https://doi.org/10.1155/2011/968031>
- Estrada-Garcia T, Lopez-Sancedo C, Zamarripa- Ayala B, Thompson MR, Gutierrez L (2004). Prevalence of *Escherichia coli* and *Salmonella spp.* in street-vended food of open markets (tianguis) and general hygienic and trading practices in Mexico City. *Epidemiology and Infection* 132(6):1181-1184.
- Evans MR, Salmon RL, Nehaul L, Mably S, Wafford L, Nolan-Farrell MZ, Gardner D, Ribeiro CD (1999). An outbreak of *Salmonella typhimurium* DT 170 associated with kebab meat and yoghurt relish. *Epidemiology and Infection* 122(3):377-383.
- Fang T, Que-Kim W, Chia-Wei L, Min-Ju H, Tzu-Hui W (2003). Microbiological quality of 18°C ready-to-eat foods products sold in Taiwan. *International Journal of Food Microbiology* 80(3):241-250.
- Feglo P, Sakyi K (2012). Bacterial contamination of street vending food in Kumasi, Ghana. *Journal of Biomedical Science* 1(1):1-8.
- Gizaw Z (2019). Public health risks related to food safety issues in the food market: A systematic literature review. *Environmental Health and Preventive Medicine* 24(1):68.
- Graphic Online (2015). <https://www.graphic.com.gh/news/general-news/unsafe-food-costs-ghana-us-69-million-annually.html#sthash.5nvBTQk6.cmf5>
- Little CL, Gillespie IA (2008). Prepared salads and public health. *Journal of Applied Microbiology* 105(6):1729-1743.
- Lopašovský L, Terentjeva M, Kunová S, Zelenáková L, Kačániová M (2016). Microbiological quality of ready-to-eat foods produced in Slovakia. *Journal of Microbiology Biotechnology and Food Science* 5(1):31-35.
- Mahami T, Odonkor ST (2012). Food Safety Risks Associated with Tertiary Students in Self Catering Hostels in Accra Ghana. *International Journal of Biology, Pharmacy and Allied Sciences* 1(4):537-550.
- Manguiat LS, Fang TJ (2013). Microbiological quality of chicken- and pork-based street-vended foods from Taichung, Taiwan, and Laguna, Philippines. *Food Microbiology* 36(1):57-62.
- Mbotto CI, Agbo BE, Ikpoh IS, Agbor RB, Udoh DI, Ambo EE, Ekim MA (2012). Bacteriological study of raw meat of Calabar Abattoir with public health and veterinary importance. *Journal of Microbiology and Biotechnology Research* 2(4):529-532.
- Meldrum RJ, Little CL, Sagoo S, Mithani V, McLaughlin J, De Pinna E (2009). Assessment of the microbiological safety of salad vegetables and sauces from kebab take-away restaurants in the United Kingdom. *Food Microbiology* 26(6):573-577.
- Mensah P, Yeboah-Manu D, Owusu-Darko K, Ablordey A (2002). Street foods in Accra, Ghana: How safe are they?. *Bulletin: World Health Organization* 80(7):546-554.
- Ministry of Food and Agriculture (MOFA)/World Bank (2007). *Review of food safety in Ghana*. Accessed (16th February, 2018).
- Monney I, Agyei D, Ewoenam BS, Priscilla C, Nyaw S (2014). Food hygiene and safety practices among street food vendors: an assessment of compliance, institutional and legislative framework in Ghana. *Food and Public Health* 4(6):306-315.
- Monney I, Agyei D, Owusu W (2013). Hygienic practices among food vendors in educational institutions in Ghana: the case of Konongo. *Foods* 2(3):282-294.
- New South Wales (NSW) Food Authority. (2009). *Microbiological quality guide for ready-to-eat foods e A guide to interpreting microbiological results*. Newington, NSW. Available from

- A Case Study from Kumasi, Ghana. WIEGO Resource Document No. 15. Manchester, UK: WIEGO.
- Ologhobo AD, Omojola AB, Ofongo ST, Moiforay S, Jibir M (2010). Safety of street vended meat products e chicken and beef 'suya'. *African Journal of Biotechnology* 9(26):4091-4095.
- Osei Mensah J, Ohene-Yankyera K, Aidoo R (2016). Constraints to growth of micro and small-scale enterprises in Ghana: A case of street food enterprises. *Journal of Development and Agricultural Economics* 8(10):241-250.
- Osei-Tutu B, Anto F (2016). Trends of reported foodborne diseases at the Ridge Hospital, Accra, Ghana: a retrospective review of routine data from 2009-2013. *BMC Infectious Diseases* 16(1):139.
- Panozzo M, Magro L, Erle I, Ferrarini S, Murari R, Novelli E, Masaro S (2015). Nutritional quality of preparations based on Döner Kebab sold in two towns of Veneto Region, Italy: preliminary results. *Italian Journal of Food Safety* 4(2).
- Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM (2009). *GenStat for Windows*. 12th Edition, VSN International, Hemel Hempstead.
- Scallan E, Griffin P, Angulo F, Tauxe R, Hoekstra R (2011b). Foodborne illness acquired in the United States-unspecified agents. *Emerging Infectious Disease* 17(1):16-22.
- Scallan E, Hoekstra RM, Angulo F, Tauxe R, Widdowson M, Roy SL, Jones JL, Griffin PM (2011a). Foodborne illness acquired in the United States-major pathogens. *Emerging Infectious Disease* 17:7-15.
- Schroeder CM, Naugle AL, Schlosser WD, Hogue AT, Angulo FJ, Rose JS (2005). Estimate of illnesses from Salmonella enteritidis in eggs, United States, 2000. *Emerging infectious Diseases* 8(10):2385-2388.
- Shaltout FA, El-Shater AH, Wafaa MA (2015). Bacteriological assessment of Street Vended Meat Products sandwiches in kalyobia Governorate. *Journal of Benha veterinary Medicine* 28(2):58-66.
- Synnott M, Morse DL, Maguire H, Majid F, Plummer M, Leicester M, Threlfall EJ, Cowden J (1993). An outbreak of Salmonella mikawasima associated with doner kebabs. *Epidemiology and Infection* 111:473-481.
- Tambekar DK, Jaiswal VJ, Dhanorkar DV, Gulhane PB, Dudhane MN (2008). Identification of microbiological hazards and safety of ready-to-eat food vended in streets of Amravati city, India. *Journal of Applied Biosciences* 7:197-201.
- Tavakoli HR (2008). *Food microbiology and control of food production and distribution centers*. 2nd edition. Tehran: Marz-e-Danesh Publication.
- Tigari H, Shalini S (2020). Socio-Economic Condition of Urban StreetFood Vendors. *Shanlax International Journal of Economics* 8(3):67-74.
- Umar AA, Sambo MN, Sabitu K, Iliyasu Z, Sufiyan MB, Hamza KL (2018). Personal and food hygiene practices among street-food vendors in Sabon-Gari local government area of Kaduna State, Nigeria. *Archives of Medicine and Surgery* 3:77-83.
- Wimalasekara SGMRL, Gunasena GDDK (2016). Microbiological quality of ready-to-eat meat based food available in temporary food outlets in Gall Face Green, Colombo, Sri Lanka. *International Journal of Agriculture Forestry and Plantation* 4:38-44.
- World Health Organization (WHO) (2017). The burden of foodborne diseases in the WHO European region. Retrieved from <http://www.euro.who.int/en/health-topics/disease-prevention/food-safety/publications/2017/the-burden-of-foodborne-diseases-in-the-who-european-region-2017>
- Yiğit V, Duran T (1997). *Institutional Nutrition Technology I* (in Turkish). Ekin Publishing, Istanbul, Turkey.

Full Length Research Paper

Microbiological profile of asymptomatic bacteriuria in pregnant women in Volta Region, Ghana

Dzifa Kofi Ahiatrogah^{1*}, Jude John Annan², Linda Aurelia Ofori¹, Benedict Awudzi³⁺, Alex Owusu-Ofori⁴, Michael Owusu^{5,6} and Augustina Angelina Sylverken^{1,6}

¹Department of Theoretical and Applied Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

²Department of Obstetrics and Gynaecology, School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

³Department of Microbiology Laboratory, Ho Teaching Hospital, Ho, Ghana.

⁴Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

⁵Department of Medical Diagnostics, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

⁶Kumasi Centre for Collaborative Research in Tropical Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, Kumasi, Ghana.

Received 3 July, 2021; Accepted 18 August, 2021

Asymptomatic bacteriuria can lead to urinary tract infections in as many as 20% of pregnant women. Asymptomatic bacteriuria in pregnancy can also lead to preterm births and low birth weights. The objective of this study was to profile uro-pathogens and describe the population-based prevalence, the antimicrobial sensitivity pattern, and ascertain the risk factors for asymptomatic bacteriuria among pregnant women attending the antenatal clinic of Ho Teaching Hospital, in Ghana. Urine samples were cultured, isolates identified and antibiotic sensitivity testing was done using the Clinical and Laboratory Standard Institute (CLSI) guidelines. 46 (13.7%) out of 335 pregnant women had asymptomatic bacteriuria. The most frequently isolated bacteria were *Pseudomonas species* (26.1%) followed by *Escherichia coli* (21.7%). All isolates (n=46; 100%) were resistant to Augmentin whereas 87% of the isolates (n=40) were susceptible to Gentamicin. However, most of the isolates were multi-resistant to antibiotic drugs. No education (p=0.019) and first trimester (p=0.046) of pregnancy were risk factors for asymptomatic bacteriuria. *Pseudomonas aeruginosa* was the most frequent organism isolated. All the uro-pathogens were resistant to Augmentin, while high rates of resistance to Tetracycline, Amikacin, Norfloxacin, and Levofloxacin were observed. The study reveals that asymptomatic bacteriuria was significantly associated with the first trimester of pregnancy and having no education.

Key words: Bacteriuria, urinary tract infections, prevalence, Ghana, Ho Teaching Hospital, antimicrobial resistance.

INTRODUCTION

Asymptomatic bacteriuria (ASB) is the presence of true bacteriuria without subjective evidence of urinary tract infection (UTI) such as dysuria, urgency, and frequency (Cortes-Penfield et al., 2018). The bacteria are persistent,

actively multiplying within the urinary tract and this can lead to infection in as many as 20% of pregnant women (Al-mijalli, 2017).

The prevalence of ASB in pregnant women is 4 to 7%

(Ghafari et al., 2016). The burden of ASB vis a vis UTI differs geographically and it is on the rise across the regions (Chamoun, 2020). Globally, the burden of UTI (progression of ASB) is estimated to be 150 million cases per year (WHO, 2005b) and it is predominantly increasing in developing countries than in developed countries (WHO, 2005a). There is also an increase in healthcare-associated infection varying between 5.7 and 19.1% and this includes asymptomatic bacteriuria (WHO, 2005a).

ASB can occur in both males and females but is predominant in females possibly because females have shorter urethra which is closer to the anal region (Abujheisha, 2020; Salvatore et al., 2011). Hence, there is easy colonization and migration of uro-pathogens to the different parts of the urinary tract (Geerlings, 2016; Van Brummen et al., 2006). Other factors that influence the occurrence, progress, and outcome of ASB in pregnancy are the anatomical and physiological changes of the renal system during pregnancy (Cheung and Lafayette, 2013; Easmon et al., 1985; Oli et al., 2011). These changes include the kidneys, renal pelvis, and calyces becoming larger, and ureters dilate markedly during pregnancy (right>left) which is thought to be progesterone-induced (Faiz et al., 2020). There is also increased vesicoureteral reflux and occurrence of urinary stasis or hydronephrosis in the ureters which predisposes pregnant women to asymptomatic bacteriuria to develop symptomatic pyelonephritis (Cietak and Newton, 1985; Oli et al., 2010).

Meanwhile, the colonization of the vagina region by these pathogenic microorganisms associated with ASB if persist have a direct bearing on both the health of the woman and the pregnancy (Debaun et al., 1993; Kline and Lewis, 2016; Sheikh et al., 2000). The uro-pathogen of ASB are predominantly the Gram-negative bacteria and *E. coli* is reported to be the dominant of them (Foxman, 2003; Tabibian et al., 2008; Tupin et al., 2007). *Enterococcus spp*, *Citrobacter spp*, *Proteus spp*, *Pseudomonas aeruginosa*, *Klebsiella spp*, and *Acinetobacter spp*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and *Staphylococcus epidermidis* are some of the other frequently occurring bacteria implicated in UTI (Tabibian et al., 2008).

It is, however, reported that about 8% of pregnant women may have experienced urinary tract infection and recurrent urinary tract infection (RUTI) in the third trimester which is a result of the progression of untreated asymptomatic bacteriuria (Ahmed and Ghadeer, 2013). In Ghana, the prevalence and the antimicrobial susceptibility pattern of ASB among pregnant women varies from area to area and there is no published work on ASB among pregnant women in Ho, Volta Region.

There is also the need for updated information on ASB, the bacterial etiologies associated with ASB, and the use of antimicrobial agents in different settings. Hence, the purpose of the study was to characterize uro-pathogens and describe the antimicrobial sensitivity pattern among pregnant women attending the antenatal clinic of Ho Teaching Hospital.

MATERIALS AND METHODS

Study design

The authors conducted a cross-sectional study on pregnant women attending Ho Teaching Hospital, Ho, Ghana from July, 2019 to January, 2020. The study was designed to isolate and identify the bacteria in the urine of pregnant women attending the antenatal clinic of the Hospital and determine their susceptibility to commonly used antibiotics in the hospital.

Study site

The study was conducted at the Ho Teaching Hospital (HTH) in the Volta Region of Ghana. The hospital serves as the central point to health care and referral in the Volta and Oti Regions of Ghana and some parts of the Republic of Togo, and Benin. Volta Region is composed of 17 districts with a population of 1,907,679 and the Oti Region has a population of 759,799 with 8 districts (Ghana Statistical Service, 2020).

Study Population

The study included 335 pregnant women attending the Antenatal Clinic (ANC) at the Ho Teaching Hospital only.

Sample size determination and sampling techniques

A single proportion formula ($n = z^2 p (1-p) / d^2$) was used to calculate the sample size, arriving at 103 participants. Where z represented Z score for 95% confidence interval = 1.96, p = prevalence, and d = acceptable error (5%). (Kish, 1965) A prevalence, 7.3%, of asymptomatic bacteriuria in pregnant women attending Komfo Anokye Teaching Hospital was used in calculating the sample size since no similar work has been done in the study area. (Turpin et al., 2007).

$$\begin{aligned} n &= z^2 p (1-p) / d^2 \\ &= (1.95)^2 \times (0.073) (1-0.073) / (0.05)^2 \\ &= 103 \end{aligned}$$

However, the desired 103 participants were extrapolated to 335 clients until the reagents used were finished.

Inclusion criteria

All pregnant women attending the antenatal clinic at HTH.

*Corresponding author. E-mail: dzifaahiatrogah4@gmail.com, dkahiatrogah@st.knust.edu.gh.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

Exclusion criteria

Pregnant women with a history of antibiotic treatment in the last month were excluded since it is likely not to have bacteria in their urine. Also, pregnant women with any form of vaginal discharge and bleeding were excluded from the study.

Sample and data collection

Closed-ended questionnaires were administered to study participants satisfying the inclusion criteria to obtain information on their socio-demographic, obstetric characteristics of the pregnancy, gestation age, knowledge on UTI, knowledge on antibiotics, clinical history of UTI and antibiotics, and personal hygiene. The participants were enrolled at the antenatal clinic.

Bacteriological investigation

Sterile universal containers were given to participants after completing the questionnaires on the day of enrolment. Study participants were taught and instructed on the correct mode of self-collection of a fresh morning midstream urine.

These containers were labeled with their respective codes, date, and time of collection after the urine samples were received at the laboratory. The urine samples were cultured on a cysteine lactose electrolyte deficient (CLED) agar plate using a 0.002mL calibrated loop. These plates were incubated aerobically overnight in an aerobic incubator. After 24-48 hours, the plates were read as; significant bacterial growth, no significant bacteria growth, or no bacterial growth. Bacteriuria is determined as the approximate number of bacteria per ml of un-centrifuged urine estimated using a sterile special calibrated wire loop (that can hold 1/500 mL that is 0.002 mL of urine) for inoculation on sterile culture medium and incubated aerobically at 35°C to 37°C for 24-48 h. The number of isolated colonies (Colony forming units) on the agar medium is then counted using a counting chamber and then multiplied by a factor of 500 to estimate significant bacteriuria. A count of more than 10^5 per mL of urine is taken as significant bacteriuria; less than 10^4 per mL is taken as not significant while counts between 10^4 - 10^5 per ml were considered doubtful and the urine samples were re-examined. High colony counts with mixed growths of species were considered as contamination. The significant bacteria growth showed in the urine indicates the potential of asymptomatic bacteria since infected pregnant women were not showing signs and symptoms of UTI.

Identification of significant bacterial growth was done using colony morphology, microscopic, and biochemical tests (Murray et al., 1995). The Gram reactions were determined by the use of gram staining.

Standard biochemical identification of bacterial isolates

Biochemical identification of bacterial isolates in the study was done based on the Clinical and Laboratory Standard Institute (CLSI) guidelines. (CLSI, 2019) Coagulase and catalase tests were used to identify Gram-positive organisms while indole, urease, citrate, triple sugar iron (TSI), oxidase tests were used in the cases of Gram-negative rods.

Confirmed bacteria strains (uro-pathogens) were then sub-cultured on the CLED medium to achieve absolute pure colonies. These uro-pathogens were emulsified into a cryovial tube containing 5 mL of 15% glycerol and 85% Brain-Heart infusion broth and stored in the freezer at -20°C.

Antimicrobial susceptibility testing (AST)

AST was done using the Kirby Bauer disc diffusion following the Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2019). Using sterile swabs, suspensions (inoculum) of 0.5 Mcfarland were seeded on sterile Mueller Hinton agar and allowed to dry for 10-15mins. Antibiotic discs were gently placed and pressed down on the agar using sterile forceps. The plates were inverted and incubated for 18-24 h. The zone diameters of the various antibiotics were measured using a straight rule. The antibiotics used included Augmentin (30 µg), Ciprofloxacin (5 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Piperacillin (20 µg), Amikacin (30 µg), Nitrofurantoin (300 µg), Nalidixic Acid (30 µg), Ceftazidime (20 µg), Norfloxacin (20 µg), Tetracycline (30 µg) and Levofloxacin (5 µg).

Quality control

Strict measures were considered in the data collection, processes, and analyses. Culture media sterility tests and performance tests were conducted on the media with known organisms as positive control and no organism as a negative control. The control organisms used were *E.coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 10237. All laboratory tests and analyses were carried out per the CLSI (2019) standard operating procedures.

Data management

Data gathered were entered into Excel and exported into R Studio software (R version 3.6.0) and analyzed. The Chi-square test at a 95% significance level was used to establish statistical associations between categorical variables and risk factors. Probability values of < 0.05 indicated a statistical relationship between the categorical variables. Descriptive statistics such as prevalence, proportions, frequencies, and ratios were used to provide summaries of the data in the study. Cross tabulation and table creations were used to construct a contingency table of the risk factors at each combination level that is to enhance understanding of the relationship between two variables.

Ethical approval

Facility approval was given by the HTH whereas the ethical approval was by the Committee on Human Research, Publication, and Ethics of the Komfo Anokye Teaching Hospital, and Kwame Nkrumah University of Science and Technology, Kumasi (CHRPE/AP/429/19 – 9th July, 2019). Participants gave their written consent and were given the right to either continue or withdraw from the study as and when necessary. However, for each confirmed case of infection, the clinician of that participant was informed and provided with the result of the antimicrobial susceptibility tests for treatment. Also, other relevant information obtained at each level of the study was kept confidential.

RESULTS

Socio-demographic characteristics of participants

The age of pregnant women in the study ranged from <20 to 49. The ages <20 and 40-49 had the highest

Table 1. Socio-demographic characteristics of the study population associated with bacteriuria (N=335).

Variable	Response	No of Participants	No of participants with ASB (%)	p-value
Gestation period	1st	76	13 (17.10)	0.046
	2nd	112	15 (13.39)	
	3rd	147	18 (12.24)	
Age	<20	12	2 (16.67)	0.777
	20-29	160	25 (15.62)	
	30-39	157	18 (11.46)	
	40-49	6	1 (16.67)	
Educational Level	No education	34	8 (23.53)	0.019
	Basic	108	13 (12.04)	
	Secondary	74	11 (14.86)	
	Tertiary	119	14 (11.76)	
Occupation	Banker	3	1 (33.33)	0.995
	Nurse	13	0	
	Teacher	36	7 (19.44)	
	Trader	96	14 (14.58)	
	Other	187	24 (12.83)	
Marital Status	Married	285	39 (13.68)	0.135
	Separated	5	1 (20)	
	Single	45	6 (13.33)	
	Divorced	0	0	
	Widowed	0	0	
Knowledge on UTI	Yes	276	7 (2.53)	0.473
	No	59	39(66.10)	
Presence of Toilet Facility	Yes	277	38 (13.71)	0.460
	No	58	8 (13.79)	

bacteriuria percentage (n=2:1; 16.67%) and the age range 30 - 39 recorded the lowest number of bacteria isolates (n=18; 11.46%). There were no significant associations between age (p=0.777), marital status (p=0.135), and occupation (p=0.995) with bacteriuria but a significant association was established between the gestation period (p=0.046), and educational level (p=0.019) with bacteriuria. Although 277 out of the 335 study participants had toilet facilities in their homes, the rates were the same whether patients had toilet facilities at home or not (Table 1).

Distribution of isolates

The 335 pregnant women enrolled in this study 46 had asymptomatic bacteriuria giving a prevalence of bacteriuria to be 14%. 75 (22%) showed no significant

growth (NSG), while 214 (64%) showed no bacteria growth (NBG) (Figure 1).

Frequencies and type of isolates

Thirteen (n=13) uro-pathogens were isolated from the urine of the study participants with Enterobacteriaceae being the group with the most isolated organisms. Bacteria detected were *Acinetobacter species*, *Citrobacter koseri*, *Enterococcus species*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella species*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas species*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*. *Candida species* was the only fungus isolated. The most frequently detected bacteria species was *Pseudomonas species* (n=12; 26.09%) then *Escherichai coli* (n=10; 21.74%) while

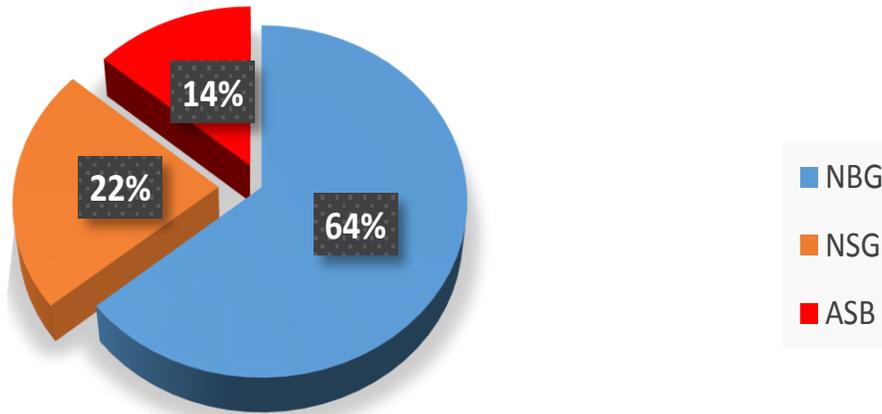


Figure 1. Distribution of Isolates in the urine of pregnant women. *NSG-Non-significant growth, NBG-No Bacteria growth, ASB-asymptomatic bacteriuria.

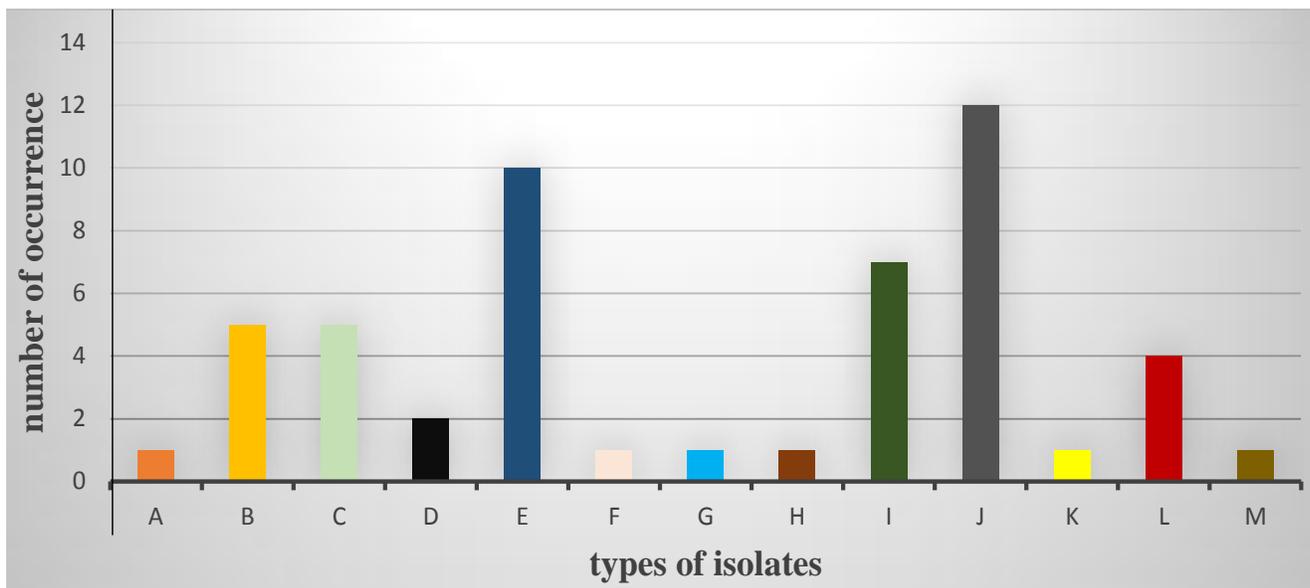


Figure 2. Frequency and types of isolates. A=Acinetobacter species, B=Citrobacter koseri, C=Candida species D=Enterococcus species, E=Esherichia coli, F=Klebsiella oxytoca, G=Klebsiella species, H=Proteus vulgaris, I=Pseudomonas aeruginosa, J=Pseudomonas species, K=Staphylococcus aureus, L=Staphylococcus epidermidis and M=Staphylococcus saprophyticus.

Acinetobacter species, *Klebsiella oxytoca*, *Klebsiella species*, *Proteus species*, *Staphylococcus aureus*, and *Staphylococcus saprophyticus* were the least detected (n=1; 2.17% each) (Figure 2).

Antimicrobial susceptibility pattern of bacterial uropathogens

Bacteria strains from the participants showed susceptibility to Gentamicin (n=40; 87%) and highest resistance to Augmentin (n=46; 100%) (Figure 3). *Pseudomonas species* showed significant resistance to Augmentin, Ceftazidime, Tetracycline, Amikacin,

Norfloxacin, Levofloxacin, and Nalidixic Acid. Also, most of the isolated organisms were susceptible to Amikacin, Ceftazidime, Ciprofloxacin, Nitrofurantoin, and Piperacillin (Table 2). *Klebsiella species*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Enterococcus species*, *Acinetobacter species*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* were either intermediate or resistant to all the antibiotics used.

Color of urine associated with bacterial urinary tract infections

245 (73.13%) urine samples were amber-colored

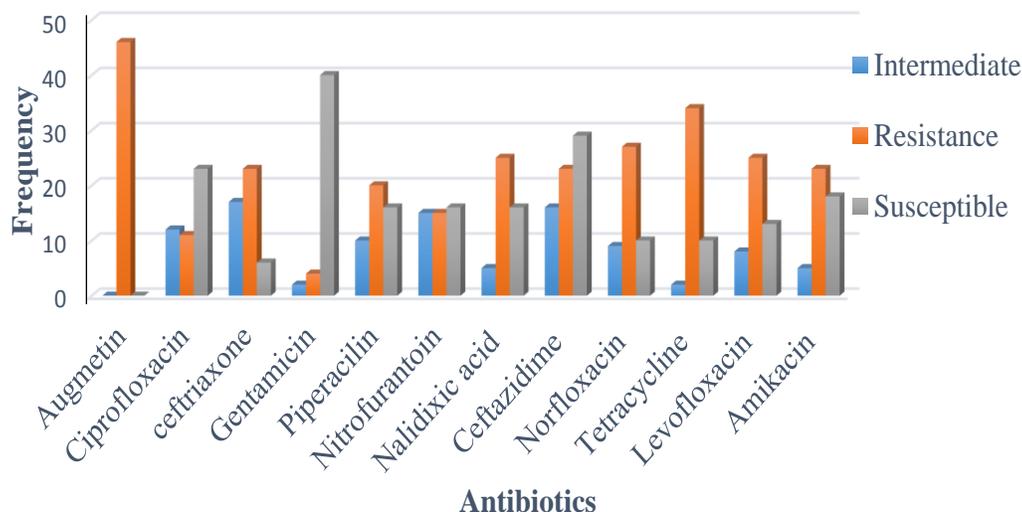


Figure 3. Antibiotic susceptibility of bacteria strains

representing the highest of the colored urine identified. Clear and transparent yellow-colored urine was the least ($n=3$; 0.89%) of the colored urine. However, straw-colored urine had the highest number of bacteria ($n=16$; 21.91%) whereas amber-colored urine recorded the least number of bacteria ($n=33$; 13.47%). Meanwhile, clear and transparent yellow-colored urine showed no bacteria growth. Nonetheless, there was no association between the color of urine and uro-pathogens detected (Chi square=32.141; $df=4$; $p=0.996$) (Table 3).

DISCUSSION

Asymptomatic bacteriuria has remained one of the most common infections diagnosed using culture sensitivity (Gebremariam et al., 2019; Yusuf et al., 2015). The overall prevalence rate of bacteria growth in the urine of pregnant women attending Ho Teaching Hospital is 13.7% and comparably higher than similar studies conducted in other hospitals in Ghana such as; the Komfo Anokye Teaching Hospital (7.3%), the University Hospital, Kumasi (9.5%), and the Korle Bu Teaching Hospital (5.5%) (Labi et al., 2015; Obirikorang et al., 2012; Tupin et al., 2007). The prevalence, however, was lower than similar studies conducted at the Cape Coast Teaching Hospital (56.5%) and the Ghana Police Hospital (31.6%) (Boye et al., 2012; Gyansa-Lutterodt et al., 2014). Other studies conducted in Saudi Arabia, Uganda, Sudan, and Nigeria reported a higher prevalence of ASB among pregnant women (Al-mijalli, 2017; Andabati and Byamugisha, 2009; Ezeome et al., 2006; Hamdan et al., 2011; Oli et al., 2011) whereas studies conducted in United Arab Emirate and Northwest Ethiopia showed lower prevalence (Abdullah and Al-Moslih, 2005; Alemu et al., 2012). The varying prevalence

among these studies is a result of the varying population characteristics including age, educational level, genital and personal hygiene, socioeconomic status and habits of the community, health care during pregnancy, and sexual activities.

The present study recorded six (6) pregnant women within the age group of 40-49. One (1) out of the six pregnant women was positive for asymptomatic bacteriuria and was one of the highest percentages of bacteria isolates. The age range <20 also showed an equal percentage of bacteria isolates as the age group 40-49 (Table. 1). Both age groups happen to be the highest bacterial growth age groups. A review conducted to profile uro-pathogens among pregnant women in Denmark reported the age group of pregnant women <25 to have the highest bacteriuria (Greve et al., 2020). A similar review conducted in the United States reported that pregnant women within the extreme age groups (<20 and >45) should have the utmost clinical attentions because these pregnant women are at risk of premature birth, cesarean deliveries, stillbirth defects, preeclampsia, and infections (Cavazos-rehg et al., 2016). The reviews corroborated the findings of this study and however, put forward that knowledge about the ages of pregnant women that are more susceptible to bacteriuria in a respective locality will be important in enhancing and improving diagnosis and clinical attention.

The variations among the trimesters with the highest ASB could be attributed to environmental factors, cultural settings and not using the same standard operating protocols across the different studies. This study demonstrated that pregnant women in the first trimester had the highest bacteriuria percentage. However, previous studies reported the second trimester to record the highest bacterial growth (Boye et al., 2012; Kehinde et al., 2011; Masinde et al., 2009; Obirikorang et al., 2012).

Table 2. Sensitivity pattern of the various antibiotics used.

Drugs	<i>Pseudomonas spp. (%)</i>			<i>E. coli (%)</i>			<i>Pseudomonas aeruginosa (%)</i>			<i>Citrobacter koseri (%)</i>			<i>Staphylococcus epidermidis</i>		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
AUG	0	0	12(100)	0	0	9(100)	0	0	7(100)	0	0	5(100)	0	0	4(100)
CIP	6(50)	1(8)	5(42)	4(44)	3(33)	2(22)	4(57)	0	3(43)	3(60)	1(20)	1(20)	4(100)	0	0
CFT	2(17)	5(42)	5(42)	2(22)	4(44)	3(33)	2(29)	1(14)	4(57)	0	1(20)	4(80)	0	1(25)	3(75)
GEN	11(92)	0	1(8)	8(89)	0	1(11)	7(100)	0	0	4(80)	0	1(20)	2(50)	1(25)	0
PIP	5(42)	4(33)	3(25)	3(33)	1(11)	5(56)	4(57)	2(29)	1(14)	0	1(20)	4(80)	2(50)	2(50)	0
CEF	1(8)	5(42)	6(50)	2(22)	3(33)	4(44)	1(14)	3(43)	3(43)	0	0	5(100)	1(25)	2(50)	1(25)
NIT	3(25)	3(25)	6(50)	4(44)	3(33)	2(22)	2(29)	3(43)	2(29)	1(20)	0	4(80)	2(50)	2(50)	0
NAL	5(42)	1(8)	6(50)	4(44)	0	5(56)	3(43)	1(14)	3(43)	2(40)	0	3(60)	1(25)	1(25)	2(50)
LEV	4(33)	2(17)	6(50)	3(33)	1(11)	5(56)	1(14)	2(29)	4(57)	1(20)	1(20)	3(60)	2(50)	1(25)	1(25)
TET	2(17)	0	10(83)	2(22)	0	7(78)	2(29)	1(14)	4(57)	0	0	5(100)	3(75)	0	1(25)
NOR	3(25)	3(25)	6(50)	3(33)	3(33)	3(33)	1(14)	0	6(86)	1(20)	0	4(80)	0	1(25)	3(75)
AMK	1(8)	1(8)	10(83)	7(78)	0	2(22)	1(14)	1(14)	5(71)	3(60)	1(20)	1(20)	3(75)	1(25)	0

AUG=Augmentin, AMK=Amikacin, CIP=Ciprofloxacin, CEF=Ceftazidime, CFT=Ceftriaxone, GEN=Gentamicin, LEV=Levofloxacin, NAL=Nalidixic Acid, NIT=Nitrofurantoin, NOR=Norfloxacin, PIP=Piperacillin, and TET=Tetracycline.

Table 3. Urine color and Uro-pathogen prevalence in pregnant women.

Color of urine	No. of pregnant women (%)	No. of Uro-pathogens (%)	P-Value
Amber	245 (73.13)	33 (13.47)	0.996
Clear	3 (0.89)	0	
Cloudy	11 (3.28)	2 (18.18)	
Straw	73 (21.79)	16 (21..91)	
Transparent yellow	3 (0.89)	0	

$\chi^2 = 32.141$; degree of freedom (df) =4; p= 0.996.

Elsewhere, a study conducted in Southern Ethiopia reported both the second and third trimesters recording the highest rate of asymptomatic bacteriuria (ASB) (Tadesse et al., 2014). A study conducted to investigate the magnitude of ASB on Indian pregnant women reported that pregnant women in their third

trimesters presented with ASB more than pregnant women in their first and second trimesters (Bhavana et al., 2019). On the other hand, the high prevalence of ASB in the first trimester of this study may be attributed to the knowledge base of the pregnant woman about the causes and preventions of ASB. This is on the assumption

that pregnant women in their second and third trimesters may have experienced one or more episodes of ASB and have been educated accordingly.

There was a significant association between educational level and ASB. This is, however, in variance with previous studies conducted across

the world that reported a significant association between socioeconomic status (education status) and ASB (Alemu et al., 2012; Gilstrap and Ramin, 2001; Masinde et al., 2009; Oli et al., 2010). Nonetheless, this assertion aligns with a study conducted in the largest hospital in Ghana to measure the prevalence of ASB among antenatal patients and associated risk factors (Labi et al., 2015). This disparity suggests the importance of educating all categories of pregnant women about the dangers, causes, and preventions of ASB.

This study further indicates the different uro-pathogens isolated from the urine of the study participants with Enterobacteriaceae being the largest group of the isolated organisms. *Pseudomonas species* was the most frequently isolated bacterium which is contrary to most studies across the globe that reported *E. coli* as the dominant organism isolated (Getachew, 2010; Gilstrap and Ramin, 2001; Masinde et al., 2009; Obiogbolu et al., 2009). *Pseudomonas species* are mostly among the pathogens responsible for hospital-acquired infections (HAI) which includes urinary tract infections. *Pseudomonas species* are mostly found in a moist environment and on the skin of some people which can cause infection in immunocompromised individuals. Notably, during pregnancy, the pregnant woman's immune system changes, and the moist nature of the vagina opening promotes the likelihood of the pregnant women experiencing some infections including pseudomonas infection (ASB). There is, therefore, a high possibility of increased pseudomonas infection hence the plausible explanation for the high number of *pseudomonas species*.

Pseudomonas species and *Pseudomonas aeruginosa* respectively recorded a 25 and 29% susceptibility to Nitrofurantoin. They were also susceptible to Gentamicin and Ciprofloxacin. This is in agreement with other studies that reported most of their isolates including *Pseudomonas species* and *Pseudomonas aeruginosa* to be susceptible to Gentamicin, Ciprofloxacin, and Nitrofurantoin (Blomberg et al., 2005; Masinde et al., 2009). *Klebsiella species*, *Klebsiella oxytoca*, and *Acinetobacter species* showed alarming resistance to most of the antimicrobial agents used in this study. Although the sample size of the study was relatively small it can, however, be deduced that *Klebsiella species*, *Klebsiella oxytoca*, and *Acinetobacter species* are emerging as a multidrug-resistant organism in the study since they were resistant to all antibiotics used for the antimicrobial sensitivity tests (AST). This could also be attributed to the abilities of these isolates to naturally encode genes that are resistant determinants and by accumulating multiple mechanisms of resistance which lead to the development of pan-resistant strains (Bonomo and Szabo, 2006). There have been similar studies across the world including regions of Africa that agree with the increasing number of *Klebsiella species* becoming multidrug-resistant (Afriyie et al., 2014;

Leopold et al., 2014; Moran et al., 2005; Van der Bij and Pitout, 2012).

Farrugia et al. (2012) reported that the straw color of the urine shows dehydration and the cloudy urine color indicates UTI, increased cells, or chronic disease. In harmony with the findings of Farrugia et al. (2012), this present study showed the highest bacterial growth in straw and cloudy colored urine indicating that there have been more dehydration and UTI respectively among pregnant women.

Future studies should include the use of a PCR diagnostic tool since it will target the DNA to identify antimicrobial-resistant conferring genes in the isolates. Also, subsequent studies should ascertain the prevalence of candida, rickettsia, chlamydia, and mycoplasma in pregnant women and determine the burden of candiduria among pregnant women in Ho Teaching Hospital.

Conclusion

In conclusion, *Pseudomonas aeruginosa* was the most frequent organism isolated. All the uro-pathogen were resistant to Augmentin, while most were resistant to Tetracycline, Amikacin, Norfloxacin, and Levofloxacin, hence should not be recommended unless they are efficacious against that particular isolate. However, Nitrofurantoin, Cefadizime, and Gentamicin respectively should be used as the first-line medications for women with asymptomatic bacteriuria in the Volta Region and Oti Region because they showed significant susceptibility. The study also concludes that there are significant associations between gestation periods where the first trimester respondents showed the highest percentage of bacteriuria. The educational level of pregnant women also showed a significant association with bacteriuria. It was concluded that those who received a certain level of education recorded a lower percentage of bacteriuria as compare to the high percentage of bacteriuria among uneducated respondents. Conversely, variables such as occupation, age, and marital status did not show any significant relationships with bacteriuria during this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors are grateful to the study participants, and the staff of Ho Teaching Hospital, the Laboratory Department, and the Antenatal Clinic for permitting them to use their facilities, and their contribution toward the completion of this work. They also express their gratitude to families and friends for their stimulus support.

REFERENCES

- Abdullah AA, Al-Moslih MI (2005). Prevalence of asymptomatic bacteriuria in pregnant women in Sharjah, United Arab Emirates. *Eastern Mediterranean Health Journal* 11:1045-1052.
- Abujheisha KY (2020). Asymptomatic bacteriuria in type II diabetics and non-diabetics; Risk factors, bacterial types and their corresponding resistance pattern. *Journal of Renal Injury Prevention* 9(1):5-7.
- Afriyie D, Gyansa-Lutterodt M, Asare G (2014). Antimicrobial Use and Susceptibility Pattern of Uropathogens Associated with Urinary Tract Infections at the Ghana Police Hospital Trace elements View project Antimicrobial Resistance of Salmonella Associated With Humans and Poultry in Ghana View project. *Global Journal of Pharmacology* <https://doi.org/10.5829/idosi.gjp.2014.8.3.83193>
- Ahmed A-B, Ghadeer A-S (2013). Recurrent Urinary Tract Infections Management in Women. *Sultan Qaboos University Medical Journal* 13:359-367.
- Al-mijalli SHS (2017). Bacterial Uropathogens in Urinary Tract Infection and Antibiotic Susceptibility Pattern in Riyadh Hospital, Saudi Arabia. *Cellular & Molecular Medicine* 3(1):1-6.
- Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B (2012). Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. *BMC Research Notes* DOI: 10.1186/1756-0500-5-197.
- Andabati G, Byamugisha J (2009). Microbial aetiology and sensitivity of asymptomatic bacteriuria among ante-natal mothers in Mulago hospital, Uganda. *African Health Sciences* 10(4):349-352.
- Bhavana AM, Kumari PHP, Mohan N, Chandrasekhar V, Vijayalakshmi P, Manasa RV (2019). Bacterial vaginosis and antibacterial susceptibility pattern of asymptomatic urinary tract infection in pregnant women at a tertiary care hospital, Visakhapatn, India. *Iranian Journal of Microbiology* 11(6):488-495.
- Blomberg B, Olsen BE, Hinderaker SG, Langeland N, Gasheka P, Jureen R, Kvale G, Midtvedt T (2005). Antimicrobial resistance in urinary bacterial isolates from pregnant women in rural Tanzania: Implications for public health. *Scandinavian Journal of Infectious Diseases* 37(4):262-268.
- Bonomo RA, Szabo D (2006). Mechanisms of Multidrug Resistance in *Acinetobacter* Species and *Pseudomonas aeruginosa*. *Clinical Infectious Diseases* 43(Supplement 2):S49-S56.
- Boye A, Siakwa PM, Boamong JN, Koffuor GA, Kwabena R, Ephraim D, Amoateng P, Obodai G, Penu D (2012). Asymptomatic urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana. *E3 Journal of Medical Research* 1(6):074-083.
- Cavazos-rehg PA, Krauss MJ, Spitznagel EL, Bommarito K, Madden T, Olsen MA, Peipert JF, Bierut LJ (2016). Maternal age and risk of labor and delivery complications. *Maternal and Child Health Journal* 19(6):1202-1211.
- Chamoun MN (2020). Uropathogenic *Escherichia coli* colonization of the urinary tract: pathways to chronicity. <http://hdl.handle.net/10072/396143>
- Cheung KL, Lafayette RA (2013). *Renal Physiology of Pregnancy*. *Advances in Chronic Kidney Disease* 20(3):209-214.
- Cietak KA, Newton JR (1985). Serial quantitative maternal nephrosonography in pregnancy. *British Journal of Radiology* 58(689):405-413.
- CLSI (2019). M100-S11, Performance standards for antimicrobial susceptibility testing. *Clinical Microbiology Newsletter* 23(6):49.
- Cortes-Penfield N, Trautner BW, Jump R (2018). Urinary Tract Infection and Asymptomatic Bacteriuria in Older Adults. *Physiology & Behavior* 176(1):139-148.
- Debaun M, Rowley D, Province M, Stockbauer JW, Cole SF (1993). Selected Antepartum Medical Complications and Very-Low-Birthweight Infants among Black and White Women. *American Journal of Public Health* 84(9):1495-1497.
- Easmon CSF, Hastings MJG, Neill J, Bloxham B, Rivers RPA (1985). Is group B streptococcal screening during pregnancy justified? *BJOG: An International Journal of Obstetrics & Gynaecology* 92(3):197-201.
- Ezeome IV, Ikeme AC, Okezie OA, Onyebueke EA (2006). Asymptomatic bacteriuria in pregnant women. In *Bahrain Medical Bulletin* 33(4):192.
- Faiz S, Zaveri MP, Perry JC, Schuetz TM, Cancarevic I (2020). Role of Antibiotic Prophylaxis in the Management of Antenatal Hydronephrosis, Vesicoureteral Reflux, and Ureterocele in Infants. *Cureus* 12(7).
- Farrugia KJ, Bandey H, Bleay S, Nicdæ N (2012). Chemical enhancement of footwear impressions in urine on fabric. *Forensic Science International* 214(1-3):67-81.
- Foxman B (2003). Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs. *Disease-a-Month* 49(2):53-70.
- Gebremariam G, Legese H, Woldu Y, Araya T, Hagos K, Gebreyesuswasihun A (2019). Bacteriological profile, risk factors and antimicrobial susceptibility patterns of symptomatic urinary tract infection among students of Mekelle University, northern Ethiopia. *BMC Infectious Diseases* 19(1):1-11.
- Geerlings SE (2016). Clinical presentations and epidemiology of urinary tract infections. *Urinary Tract Infections: Molecular Pathogenesis and Clinical Management* pp. 27-40.
- Getachew T (2010). Bacterial pathogens implicated in causing urinary tract infection (uti) and their antimicrobial susceptibility pattern in Ethiopia. *Patógenos causantes de infección del tracto urinario (ITU) y sus patrones de susceptibilidad antimicrobiana en Etiopía*.
- Ghafari M, Baigi V, Cheraghi Z, Doosti-Irani A (2016). The prevalence of asymptomatic bacteriuria in Iranian pregnant women: A systematic review and meta-Analysis. *PLoS One* 11(6):1-10.
- Ghana Statistical Service (2020). <https://www.statsghana.gov.gh>
- Gilstrap LC, Ramin SM (2001). Urinary tract infections during pregnancy. *Obstetrics and Gynecology Clinics of North America* 28(3):581-591.
- Greve VH, Greve T, Helmig RB (2020). Bacteriuria in pregnancy in a danish contemporary cohort of women. *Infectious Diseases in Obstetrics and Gynecology* 2020:9-12.
- Gyansa-Lutterodt M, Afriyie DK, Asare G, Amponsah SK, Abutiare H, Darko D (2014). Antimicrobial Use and Susceptibility Pattern of Uropathogens Associated with Urinary Tract Infections at the Ghana Police Hospital West Africa Postgraduate College of Pharmacists, Ghana 8(3):306-315.
- Hamdan HZ, Ziad AHM, Ali SK, Adam I (2011). Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital 2-6. *Annals of Clinical Microbiology And Antimicrobials* 10(1):1-5.
- Kehinde AO, Adedapo KS, Aimaikhu CO, Odukogbe AA (2011). Significant Bacteriuria Among Asymptomatic Antenatal Clinic Attendees In Ibadan, Nigeria 39(3):73-76.
- Kish L (1965). Survey sampling. (No. 04; HN29, K5.).
- Kline KA, Lewis AL (2016). Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. *Urinary Tract Infections: Molecular Pathogenesis and Clinical Management* pp. 459-502.
- Labi A, Yawson AE, Ganyaglo GY (2015). Prevalence and Associated Risk Factors of Asymptomatic Bacteriuria in Ante-Natal Clients in a Large Teaching. *Ghana Medical Journal* 49(3).
- Leopold SJ, Leth F, Van Tarekegn H, Schultsz C (2014). Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: a systematic review. *Journal of Antimicrobial Chemotherapy* 69(9):2337-2353.
- Masinde A, Gumodoka B, Kilonzo A, Mshana SE (2009). Prevalence of urinary tract infection among pregnant women at Bugando Medical Centre, Mwanza, Tanzania. *Tanzania Journal of Health Research* 11(3):154-159.
- Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillin-resistant *Staphylococcus aureus* in community-acquired skin infections. *Emerging Infectious Diseases* 11(6):928-930.
- Murray P, Baron E, Pfaller M, Tenover F, Tenover R (1995). *Manual of Clinical Microbiology*. American Society of Microbiology 6th edition 1482.
- Obiobolu CH, Okonko IO, Anyamere CO, Adedeji AO, Akanbi AO, Ogun AA (2009). Incidence of Urinary Tract Infections (UTIs) among pregnant women in Akwa metropolis. *Southeastern Nigeria* 4:820-824.
- Obirikorang C, Quaye L, Bio FY, Amidu N, Acheampong I, Addo K (2012). Asymptomatic Bacteriuria among Pregnant Women Attending

- An-tenatal Clinic at the University Hospital, Kumasi, Ghana. *Journal of Medical and Biomedical Sciences* 1:38-44.
- Oli A, Okafor C, Ibezim E, Akujiobi C, Onwunzo M (2011). The prevalence and bacteriology of asymptomatic bacteriuria among antenatal patients in Nnamdi Azikiwe University Teaching Hospital Nnewi; South Eastern Nigeria. *Nigerian Journal of Clinical Practice* 13(4):1-5.
- Oli AN, Okafor CI, Ibezim EC, Akujiobi CN, Onwunzo MC (2010). The prevalence and bacteriology of asymptomatic bacteriuria among antenatal patients in nnamdi azikiwe university teaching hospital nnewi; south eastern nigeria. *Nigerian journal of clinical practice* 13(4).
- Salvatore S, Salvatore S, Cattoni E, Siesto G, Serati M, Sorice P, Torella M (2011). Urinary tract infections in women. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 156(2):131-136.
- Sheikh M, Khan M, Khatoon A, Arain G (2000). Incidence of urinary tract infection during pregnancy. *Eastern Mediterranean Health Journal* pp. 265-271.
- Tabibian JH, Gornbein J, Heidari A, Dien SL, Lau VH, Chahal P, Churchill BM, Haake DA (2008). Uropathogens and Host Characteristics. *Journal of Clinical Microbiology* 46(12):3980-3986.
- Tadesse E, Teshome M, Merid Y, Kibret B, Shimelis T (2014). Asymptomatic urinary tract infection among pregnant women attending the antenatal clinic of Hawassa Referral Hospital, Southern Ethiopia. *BMC Research Notes* 7:155.
- Tupin CA, Minkah B, Danso KA, Frimpong EH (2007). Asymptomatic bacteriuria in pregnant women attending antenatal clinic at komfo anokye teach. *Ghana Medical Journal* 41(1):26-29.
- Turpin C, Minkah B, Danso K, Frimpong E (2007). Asymptomatic bacteriuria in pregnant women attending antenatal clinic at komfo anokye teaching hospital, kumasi, ghana. *Ghana Medical Journal* 41(1):26-29.
- Van Brummen HJ, Bruinse HW, Van Der Bom JG, Heintz APM, Van Der Vaart CH (2006). How do the prevalences of urogenital symptoms change during pregnancy?. *Neurourology and Urodynamics* 25(2):135-139.
- Van der Bij AK, Pitout JDD (2012). The role of international travel in the worldwide spread of multiresistant enterobacteriaceae. *Journal of Antimicrobial Chemotherapy* 67(9):2090-2100.
- World Health Organization (WHO) (2005a). Urinary Tract Infections in Infants and Children in Developing Countries in the Context of IMCI.
- World Health Organization (WHO) (2005b). *World Health Organization_2006_Child_Growth@journals.lww.com*.
http://journals.lww.com/jpgn/Abstract/2013/08000/World_Health_Organization_2006_Child_Growth.25.aspx
- Yusuf A, Begum A, Ahsan CR, Bauer K (2015). Antibiotic sensitivity pattern of gram negative uropathogenic bacilli at a private hospital in Dhaka city. *Al Ameen Journal of Medical Sciences* 8(3):189-194.

Full Length Research Paper

Goat carcass microbial investigation in Modjo Export Abattoirs, Ethiopia

Abraha Negash^{1*} and Karanfil Olga²

¹Ethiopian Meat and Dairy Industry Development Institute, Ministry of Trade and Industry, Ethiopia.

²DVM, Debrezeit, Ethiopia.

Received 18 August, 2020; Accepted 8 July, 2021

The study was conducted to investigate microbiological quality of goat carcass in the Ethiopian export abattoirs, located in Modjo from January to April, 2017. Swabbed samples of 180 were collected from three abattoirs: 144 were from 12 carcass of three anatomical sites (thorax, hind and foreleg), 24 employees' hands and apron, and 12 carcass washing water to determine coliform counts (CFU), *Escherichia coli* and total plate counts (TPC) as indicator organisms focusing carcass decontamination effects of post washing, acid spray and chilling. The mean result for TPC log/cm² was 4.22, 4.03 and 3.56 for Abattoir 1, 2 and 3, respectively. Ranging from 1.7 to 7.4 and mean 3.95 ± 1.3 TPC log/cm² for the water, employees' hands and apron. There was 1.9±1.006 TPC counts/cm², 1.38±0.874 CFU counts/cm² and 1.28±0.799 *E. coli*/cm² mean in the carcass with statistically significant difference (p<0.05) level that meat handling procedures enabled the abattoirs with minimal microbial counts from washing to chilling. Strongly significant correlation (p<.05) among the microbials was observed. The study confirmed the abattoirs slaughtering procedures enabled to deliver safe carcass with very minimum microbial counts that 96.5% of the carcass was safe cumulative wise of which 84% was categorized in excellent standards. Carcass contaminating bacteria should be determined.

Key words: Microbial, goat carcass decontamination, Ethiopian export abattoirs.

INTRODUCTION

The export of meat and meat products is an important element in the Ethiopian economy as there is an ever increasing demand for meat and meat products worldwide including beef, sheep, goat, processed meats, etc., both

as fresh and frozen products. Ethiopia has the opportunity to respond to this international demand and increase its market share in what is a very lucrative trade, subject to it meeting the stringent requirements demanded

*Corresponding author. E-mail: abrahane12@gmail.com. Tel: +251114308618

Abbreviations: APC, Aerobic plate count; SPC, standard plate counts; TPC, total plate counts; TVC, total viable counts; CFU, coliform counts; ECC, *Escherichia coli* counts.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

by livestock and meat importing countries. Because of its composition, meat of all sorts is highly susceptible to microbiological and chemical contamination. Unless derived from healthy animals, handled % processed in a proper manner, it may present a serious health hazard to consumers. To prevent this, importing countries are setting high standards for both product preparation and final product involving the most strict health and quality controls and scientific inspection. Therefore, it is essential for Ethiopia to comply with the requirements of importing countries. To do otherwise, results in costly rejections and loss of product (Nega, 2015). The safety of meat has been at the forefront of societal concerns in recent years, and indications exist that challenges to meat safety will continue in the future (Sofos, 2008).

Wholesome meat must be produced hygienically, free from pathogenic organisms and retains its natural state and nutritive value. Monitoring the prevalence of microorganisms of hygienic interest in primary production at abattoirs makes data available for effective control of pathogenic agents before they entered the food (Bhandare et al., 2010). Raw meat is an ideal medium for bacterial growth; this is due to its high moisture contents. It is rich in protein, fermentable carbohydrate (glycogen), favorable pH and other growth factors (Magnus, 1981). Mayr et al. (2003) showed that meat provides an ideal condition for the growth of different spoilage bacteria making meat very perishable. Indicator organisms are bacteria that are used to provide evidence of poor hygiene, inadequate processing or post-process contamination of foods. They are often chosen because they are relatively quick and simple to detect. Their absence in food provides a degree of assurance that the hygiene and food manufacturing process has been carried out appropriately, whereas their presence usually indicates that a potential problem or failure in the process has occurred (Mead, 2007).

Microbiological tests are important in governmental food inspection to enforce legal requirements, international trade to determine compliance with microbiological standards, commercial relationships between trading partners to ensure that agreed microbiological specifications are met, the food industry to maintain quality control and process requirements, academic areas for research purposes, and reference laboratories to confirm the results of other laboratories and to provide surveillance data (Mead, 2007). Both total viable counts (TVC) and *Escherichia coli* testing are necessary to understand the process of slaughter, dressing and chilling. Testing for other organisms may be specified by specific customer (AS 4696-2002). Testing foods and water for coliform has remained popular, not least because specific guidelines and regulations demand coliform testing. Microbiological criteria are used at any stage in the food chain to assess the acceptance of lots of raw material or finished product. They are based

on the absence/presence of certain microorganisms or quantitative limits per units. Product counts were obtained using methods set out based on surveys of Australian meat (AS 4696-2002) descriptions used: Excellent, Good, Acceptable and Marginal for microbial levels listed:

If *E. coli*/cm² is a) zero- excellent; b) 1-10= good; c) 10-100= acceptable; d) 100-1000= marginal; e) *E. coli*/cm² more than 1000 is not acceptable.

If total plate counts (TPC)/cm² is a) <1000= excellent; b) 10³-10⁴= good; c) 10⁴-10⁵= acceptable; d) 10⁵-10⁶= marginal; e) TPC/cm² more than 10⁶ is not acceptable.

If coliform counts (CFU)/cm² is a) zero= excellent; b) 1-10= good; c) 10-100= acceptable; d) 100-1000= marginal; e) *E. coli*/cm² more than 1000 is not acceptable.

Coliform more than 1000 is unacceptable. For water samples, detection of any coliform unit is undesirable.

The food industry uses microbial test as an indicator to determine the overall level of sanitation within the manufacturing and distribution processes and to determine whether the processing kill step was significant. The higher the microbial load found in TPC is, the greater is the possibility that the processing environment is not clean or that the process was not sufficient enough to kill an adequate number of the organisms present. TPC is a good indicator for the overall bacterial load of meat and meat products. Critical hygienic dimensions are reached when the total number of bacteria on fresh meat lies between 10⁴ and 10⁵/g. *E. coli* are bacteria present in intestines of human and animal. Shiga toxin-producing strains of *E. coli* or STECs are responsible for most food-related *E. coli* infections. *E. coli* O157:H7 and other STECs like *E. coli* O145 and *E. coli* O121:H19 produce a toxin called Shiga toxin, which causes illness in humans (Nagarajan et al., 2018). *E. coli* were enumerated on Eosin methylene blue agar by plating an appropriate dilution on plates followed by aerobic incubation at 37°C for 24 h. After incubation, *E. coli* were counted as colonies with distinct metallic sheen (Bhandare et al., 2007).

Objective

Therefore, the aim of the study was to investigate HACCP/GMPs practices and evaluate the microbial distribution of goat carcass at export abattoirs.

Specific objectives

(1) To satisfy regulatory requirements providing customers with information on product quality,

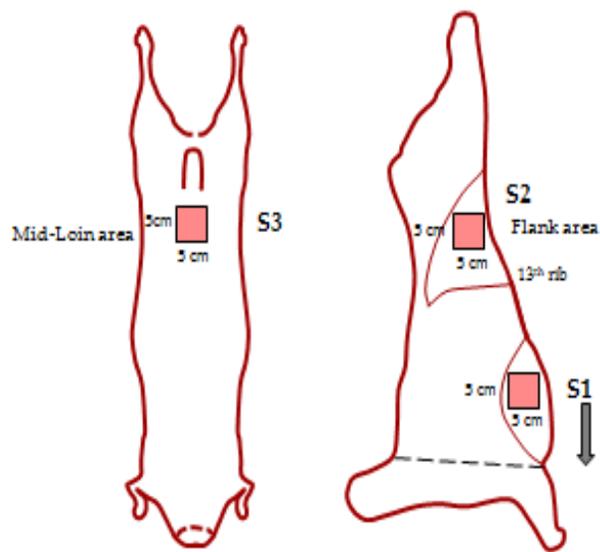
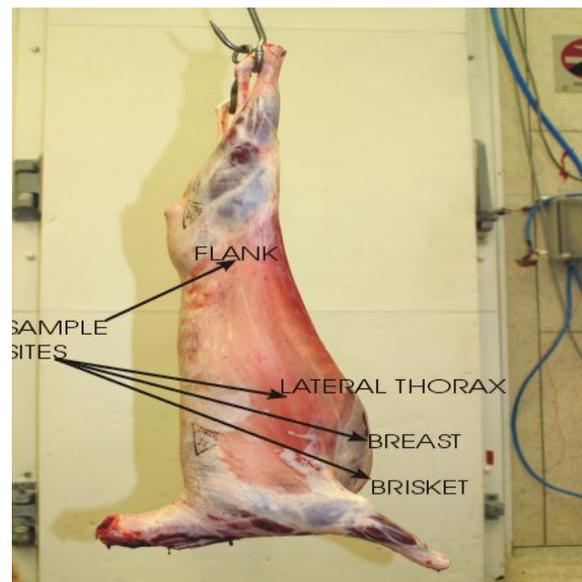


Figure 1. Sampling sites for small stock.
Source: Meat standard committee (AS 4696-2002).



- (2) To monitor process control and approve the exportable carcass quality,
- (3) To investigation poor performance with a view to improve the process of the abattoirs gauging the effectiveness of cleaning procedures,
- (4) To assess product against a national and international benchmark,

MATERIALS AND METHODS

Sampling procedures

A total number of 180 cotton swabbed samples were collected equally from three abattoirs, of which 144 were from thorax, hind and fore limbs, 12 water samples and 12 aprons and 12 employee palm. Samples were collected aseptically in sterile containers and brought to the laboratory within 30 to 45 min using ice box. After collection, bacteriological analysis of the samples were performed to assess the selected microbial attributes such as TPC, CFU and *E. coli* in goat carcass of different sources by using Plate Count (PC) agar, MacConkey (MC) agar to find out the sanitary quality of goat carcass of Ethiopian Export Abattoirs. Investigations were carried out over the period January to March, 2017, during the production of goat carcass for export. The surface to be examined was swabbed twice using parallel strokes at right angles to the first strokes. Care was taken to swab the whole of the predetermined area. After swabbing, the swabs were transferred to the respective tubes containing the 5 ml of sterile 0.1% peptone water. An area of 100 cm² marked with a sterile frame of 10 cm × 10 cm on each site of the carcass was rubbed for 30 s and swabs were transferred to a screw-capped test tube containing 10 ml of sterile maintenance medium 0.1% peptone water (Hamdan et al., 2019) (Figure 1).

The research materials included cotton swabs, ice boxes, alcohol, amenities, water, and camera and 12 samples of fresh goat

carcass were randomly assessed from 3 major export abattoirs in Modjo areas after carcass was washed and organic acid sprayed and after 24 h chilling. The samples were collected from different portions of carcasses and 36 abattoir employees' hands and apron who engaged in meat washing along with the water they used to wash. The samples were collected once/week from each export abattoir. The samples were aseptically collected in different clean polyethylene bags and were transferred immediately to the laboratory for bacteriological quality assessment. The meat samples were collected in aseptic containers labeled and transported in an ice box for 30 min, subjected to qualitative or quantitative analysis for bacterial zoonotic pathogens indicator organisms.

Statistical model for microbial count

The data on TPC, CFU and *E. coli* obtained from the study carcass surface were analyzed in completely randomized design/CRD using SPSS and Excel. Data collated were analyzed using IBM SPSS Statistics version 20 (IBM Corporation, 2011). Simple means, percentages and frequencies were computed. Means were compared using Analysis of Variance (ANOVA) and Chi-squared test was used to determine associations.

$$Y_{ijk} = \mu + T_i + S_j + (TS)_{ij} + E_{ijk}$$

where Y_{ijk} = microbial count, μ = overall mean, T_i = effect of treatment (before and after treatment), and S_j = effect of site (1 = Thorax, 2 = Hind limb and 3 = Fore limb), $(TS)_{ij}$ = interaction between treatment and site, E_{ijk} = random error.

Microbiological analysis

For food control purposes, the organisms in question are often

Table 1. CFU, *E.coli* & TPC in goat carcass with t- values and interval ranges.

Variable	Test Value=0.05					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence interval of the difference	
					Lower	Upper
CFU/cm ²	18.003	143	0.000	1.311	1.17	1.46
<i>E. coli</i> count/cm ²	18.540	143	0.000	1.235	1.10	1.37
TPC count/cm ²	22.107	143	0.000	1.853	1.69	2.02

Table 2. ANOVA for comparative mean and significance of CFU, *E.coli* & TPC of fresh carcass.

Variable		Sum of Squares	df	Mean Square	F	Sig.
CFU/cm ²	Between groups	12.500	11	1.136	0.880	0.567
	Within groups	46.500	36	1.292		
	Total	59.000	47			
<i>E. coli</i> count/cm ²	Between groups	13.562	11	1.233	1.296	0.266
	Within groups	34.250	36	0.951		
	Total	47.812	47			
TPC log/cm ²	Between groups	15.729	11	1.430	1.572	0.149
	Within groups	32.750	36	0.910		
	Total	48.479	47			

referred to as 'markers' of the microbiological quality of food, and they are seen as a key indicator/analytical tool for validating compliance with legislation. Where their occurrence in foods is associated with the possible presence of pathogens that are related to them taxonomically, physiologically and ecologically; they are termed 'index' organisms/marker. Here, it refers to any suitable organism that is deliberately added at a pre-determined level to a carcass or item of equipment in a slaughter or processing line in order to determine a possible route of cross-contamination, or to verify that a particular control measure is limiting its spread and the marker organism is one that is readily distinguished from all others present or can be isolated specifically and enumerated on a selective agar medium (Mead, 2007). Total bacterial count load is exemplified by measuring the amount heterotrophic organisms. These organisms can be tested by APC, TPC, TVC or SPC that are acronyms used fairly interchangeably by industry and testing laboratories alike, although TPC is the most common. The total aerobic plate count is useful for indicating the overall microbiological quality of a product and, thus, is useful for indicating potential spoilage in perishable products. The aerobic plate count is also useful for indicating the sanitary conditions under which the food was produced and/or processed (FAO, 2007).

The control of food safety and quality is an integral part of national programs for development. National food control systems are designed to protect the health and welfare of the consumer, to promote the development of trade in food and food products, and to protect the interests of the fair and honest food producer, processor or marketer against dishonest and unfair competition.

Microbiological examination of samples, TVCs and ECs were determined by standard plate count methods according to the criteria specified by ISO 4833:2003. Each sample homogenate swabbing was then diluted decimally in peptone water, and 1 ml aliquots were added to suitable Petri dishes, reaching a 10⁻⁶ dilution (TVC) and a 10 dilution (EC). Samples were analyzed within 24 h of

collection. The culture media were plate count agar for TVCs and violet red brilliant green agar for ECs. PCA plates were incubated at 37°C for 48 h before colonies were counted. Enterobacteriaceae were incubated at 37°C for 24 h. The detection limit was 0.50 CFU/cm² for all ovine carcasses.

Water samples 3×4=12 totally from carcass washing were collected for bacteriological analysis according to EC (2007) to estimate the number of Coliform bacilli in 100 ml of water using the presence-absence method. The method was chosen because the focus was the positive detection of *E. coli*, regardless of quantity; as the guideline for *E. coli* in drinking water is none per 100 ml, and qualitative results are sufficient for protecting public health (Nouichi, 2009; Verhille, 2013). For more than a hundred years, *E. coli* and coliform bacteria have been used as bacterial indicators of faecal pollution in water supplies. Currently, total coliform and *E. coli* are the most common microorganisms used as the primary indicator to assess water quality (Wen et al., 2020). Their use relates to the occurrence of such organisms in the faeces of man and a wide variety of warm-blooded animals, and the fact that the bacterial pathogens of greatest concern in water (Mead, 2007).

RESULTS AND DISCUSSION

The mean result for fresh goat carcass quality as determined for CFU, *E. coli* and TPC in three abattoirs of 144 samples from the three carcass sites was 1.36, 128 and 1.90 count/cm², respectively. The upper and lower values are higher for TPC than CFU and *E. coli* and it was statistically significant at p<0.05. The F-distribution is also highest for TPC (Tables 1 and 2).

The variability result of between groups due to

Table 3. Mean ± standard error of CFU, *E. coli* and TPC in Abattoirs 1, 2 and 3.

Microbial	Abattoirs	N	Mean	Std. error	95% Confidence interval for mean		Minimum	Maximum	Between-component variance	
					Lower bound	Upper bound				
Coliforms (CFU/cm ²)	1	48	1.75±1.12	0.162	1.42	2.08	1	5	0.099	
	2	48	1.17±0.69	0.100	0.97	1.37	1	5		
	3	48	1.17±0.6	0.086	0.99	1.34	1	4		
	Total	144	1.36±0.87	0.073	1.22	1.51	1	5		
	Model	Fixed effects/treatments	-	-	0.070	1.22	1.50	-		-
		Random effects/error or residual	-	-	0.194	0.52	2.20	-		-
<i>E. coli</i> count/cm ²	1	48	1.56±1	0.146	1.27	1.86	1	4	0.047	
	2	48	1.19±0.7	0.106	0.97	1.40	1	4		
	3	48	1.1±0.5	0.074	0.95	1.25	1	4		
	Total	144	1.28±0.8	0.067	1.15	1.42	1	4		
	Model	Fixed effects	-	-	0.065	1.16	1.41	-		-
		Random effects	-	-	0.141	0.68	1.89	-		-
Total Plated log/cm ²	1	48	1.77±1	0.147	1.48	2.07	1	4	-0.003	
	2	48	1.9±1	0.150	1.59	2.20	1	4		
	3	48	2.04±0.97	0.140	1.76	2.32	1	4		
	Total	144	1.9±1	0.084	1.74	2.07	1	4		
	Model	Fixed effects	-	-	0.084	1.74	2.07	-		-
		Random effects	-	-	0.084 ^a	1.54 ^a	2.26 ^a	-		-

treatments was smaller than within groups/errors or residues. Statistically remarking the population effect is small/inferior, justifying the difference could be due to chance. The F-test for TPC, *E. coli* and CFU were small (close to 1.0) indicating least effect, but comparatively higher F test values for TPC and least for CFU.

Table 3 remarked many of the descriptive statistics including the fixed and random effects of CFU, TPC and *E. coli* in abattoirs 1, 2 and 3. There is significant difference in CFU and *E. coli*

in abattoir 1 but not in TPC log/cm² mean that is reversed from abattoir 3 to 2 to 1, recommending abattoir 1 HACCP team and top management for due attention in both *E. coli* and CFU effects though comparatively safe in TPC. The research result warns abattoir 3 that scored higher TPC. There was significantly higher mean (P<0.05) in TPC/cm² (1.9±1) but least in *E. coli*/cm² (1.28±0.8) in the carcass of the abattoirs.

CFU strongly (0.54) correlate with *E. coli* and weakly correlate (0.21) with TPC. Coliform

significantly correlate with *E. coli* at P< 0.01 and P< 0.05 significant level with TPC. TPC was positively (0.13) correlated with *E. coli* weakly. So strong correlation in between coliform and *E. coli* has severe consequence in meat quality and risk in consumers' health (Table 4).

TPC log/cm² values for the water quality, employee apron and hands in the clean area are shown in Table 5 and ranged from 1.7 to 7.4 with 3.95 mean. There was no detection of CFU and *E. coli* in the carcass washing water in abattoirs 1, 2

Table 4. Correlations of Coliform, *E. coli* and Total Plate counts.

Correlation	(CFU/cm ²)	<i>E. coli</i> /cm ²	TPC log/cm ²
Coliforms (CFU/cm ²)	Pearson Correlation	0.543**	0.207 [†]
	Sig. (2-tailed)	1	0.013
	N	144	144
<i>E. coli</i> count/cm ²	Pearson Correlation		0.130
	Sig. (2-tailed)	1	0.119
	N		144
Total Plate count log/cm ²	Pearson Correlation		1

**Correlation is significant at the 0.01 level (2-tailed)
[†]Correlation is significant at the 0.05 level (2-tailed)

Table 5. Mean ± standard deviation of TPC log/cm², water quality, employee apron and hands in the clean area.

Variable	N	Minimum	Maximum	Sum	Mean
TPC log/cm ²	36	1.70	7.40	141.65	3.95±0.22

and 3, but significantly higher TPC log/cm² in abattoir 1 (3.1), abattoir 2 (2.2) and abattoir 3 (1.86), although it was categorically excellent distribution. Comparatively, in abattoir 1, there was higher bacterial contamination since both CFU and *E. coli* were in marginal category in employee apron working in clean area, followed by abattoir 2 where TPC log/cm² was marginal category that demands action for strict correction measures of HACCP implementation (Figure 3). Abattoir 1 scored the highest TPC log/cm² in washed thorax and chilled hind limb, thorax and forelimb that fall in marginal category; followed by abattoir 2 that scored higher TPC, besides to the significant difference in *E. coli* and CFU in the chilled thorax and hind limb of carcass site. Abattoir 1 scored the highest coliform and *E. coli* counts/cm² in the clean area employee apron, followed by abattoir 2 in employee swapped palm. Worker palm from clean area was higher in abattoir 3 followed by abattoir 2 that scored higher counts in the apron from clean area in TPC (Figure 2).

The study resulted in pair wise comparisons of CFU, *E. coli* and TPC in abattoirs 1, 2 and 3, and there was a significant difference between CFU and *E. coli* ($p < 0.05$). The mean difference between each pair was 0.583, 0.458 and 0.207, respectively for CFU, *E. coli* and TPC (Table 6).

The frequency of *E. coli* in the carcass was 87.5% that supported the distribution category excellent for no detection (Table 7). The maximum values for CFU/cm² and *E. coli*/cm² were 500 and 800, respectively. The excellent category out weighed in all the bacterial forms followed by good, acceptable and marginal sequentially for TPC, but for CFU, acceptable, marginal and

unacceptable were followed. The majority are located in center of normal curve (Figure 4).

When looking at the box plot, the similarities and differences of the three abattoirs in TPC distributions were striking. Comparing the interquartile ranges and quartiles that Abattoir 3 was in the lower 25 percentile and Abattoir 1 stood at the upper 75 percentile of the box plot remarking that the median (center) was roughly similar for TPC log/cm². The 25, 50 and 75% were 2.84, 3.97 and 4.7, respectively while the mean and standard deviation was 3.94±1.29. Note that the result provides more intuition about variability by interpreting small variability as stability, and large variability as lack of stability. The center of the distribution is more meaningful as a typical value for the distribution when there is little variability (little "noise") around it. In abattoirs 1 and 2, the length of the whiskers far exceeds the length of the box. A well proportioned tail would give rise to whiskers about the same length as the box, or maybe slightly longer. The box plot indicated that mean TPC log/cm² increases from abattoir 3 to 1, that the mean TPC log/cm² was 3.56, 4.03 and 4.22, respectively (Figure 5).

The carcass decontamination effects of TPC log/cm² in carcass sites displayed in comparative abattoirs 1, 2 and 3. Thoracic area after carcass washing and chilling and hind limb after chilling in abattoir 1, and hind limb after carcass washing in abattoir 2, were the highest in TPC. Figure 7 remarked that abattoir 1 leads thoracic area in many measures of carcass handling procedures that recalling caution particularly chilling room case that HACCP team should implement. There was a drop of TPC

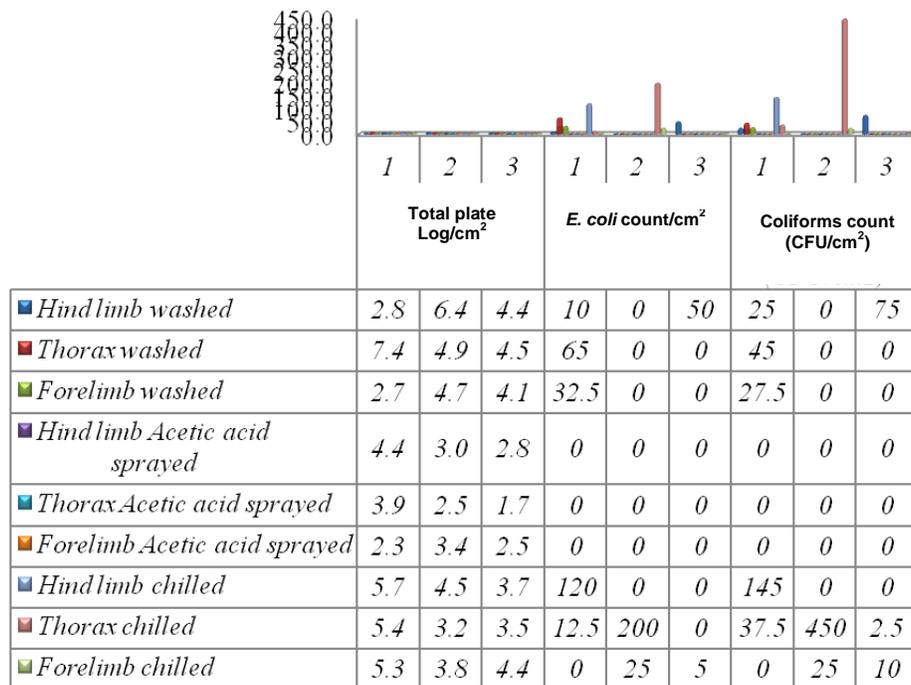


Figure 2. Microbial status of carcass sites of Abattoirs 1, 2 and 3.



Figure 3. Microbial status of water used for carcass washing and worker's apron and palm in the clean area of Abattoirs 1, 2 and 3.

load from washing to acetic acid spray to chilling slightly in thorax, hind and forelimb in abattoirs 1, 2 and 3, with the exception in hindlimb of abattoir 1 that contradictively increased from washing to acid spray and chilling, alerting HACCP team and top management for correction.

In abattoir 1, there was increasing order from washing to acetic acid spray to chilling in the hind limb (Figure 6). TPC log/cm² declined from washing to acetic acid sprayer, but slightly increased post chilling, warning chillers efficiency.

Table 6. Multiple comparisons of CFU, TPC and *E. coli* in abattoirs 1, 2 and 3.

Dependent variable		(I) Abattoirs	(J) Abattoirs	Mean difference		95% Confidence Interval		
				(I-J)	Std. error	Sig.	Lower Bound	Upper Bound
CFU/cm ²	LSD	1	2	0.583*	0.170	0.001	0.25	0.92
			3	0.583*	0.170	0.001	0.25	0.92
		2	1	-0.583*	0.170	0.001	-0.92	-0.25
			3	0.000	0.170	1.000	-0.34	0.34
		3	1	-0.583*	0.170	0.001	-0.92	-0.25
			2	0.000	0.170	1.000	-0.34	0.34
<i>E. coli</i> count/cm ²	LSD	1	2	0.375*	0.159	0.020	0.06	0.69
			3	0.458*	0.159	0.005	0.14	0.77
		2	1	-0.375*	0.159	0.020	-0.69	-0.06
			3	0.083	0.159	0.601	-0.23	0.40
		3	1	-0.458*	0.159	0.005	-0.77	-0.14
			2	-0.083	0.159	0.601	-0.40	0.23
TPC log/cm ²	LSD	1	2	-0.125	0.205	0.544	-0.53	0.28
			3	-0.271	0.205	0.190	-0.68	0.14
		2	1	0.125	0.205	0.544	-0.28	0.53
			3	-0.146	0.205	0.479	-0.55	0.26
		3	1	0.271	0.205	0.190	-0.14	0.68
			2	0.146	0.205	0.479	-0.26	0.55

*The mean difference is significant at the 0.05 level.

Table 7. Frequency value of swapped carcass part microbial counts.

Microbial type	Safety level	Frequency	Percent	Valid percent	Cumulative percent
Coliform (CFU/cm ²)	Excellent	121	84.03	84	84
	Good	1	0.69	0.7	84.7
	Acceptable	17	11.81	11.8	96.5
	Marginal	3	2	2.1	98.6
	Unacceptable	2	1.39	1.4	100
	Total		144	100.00	100
Total plate count (log/cm ²)	Excellent	67	46.53	46.5	46.5
	Good	37	25.69	25.7	72.2
	Acceptable	27	19	18.8	91
	Marginal	13	9.03	9	100
	Total		144	100.00	100
<i>E. coli</i> (count/cm ²)	Excellent	126	87.50	87.5	87.5
	Good	3	2.08	2.1	89.6
	Acceptable	7	4.86	4.9	94.4
	Marginal	8	5.56	5.6	100
	Total		144	100.00	100

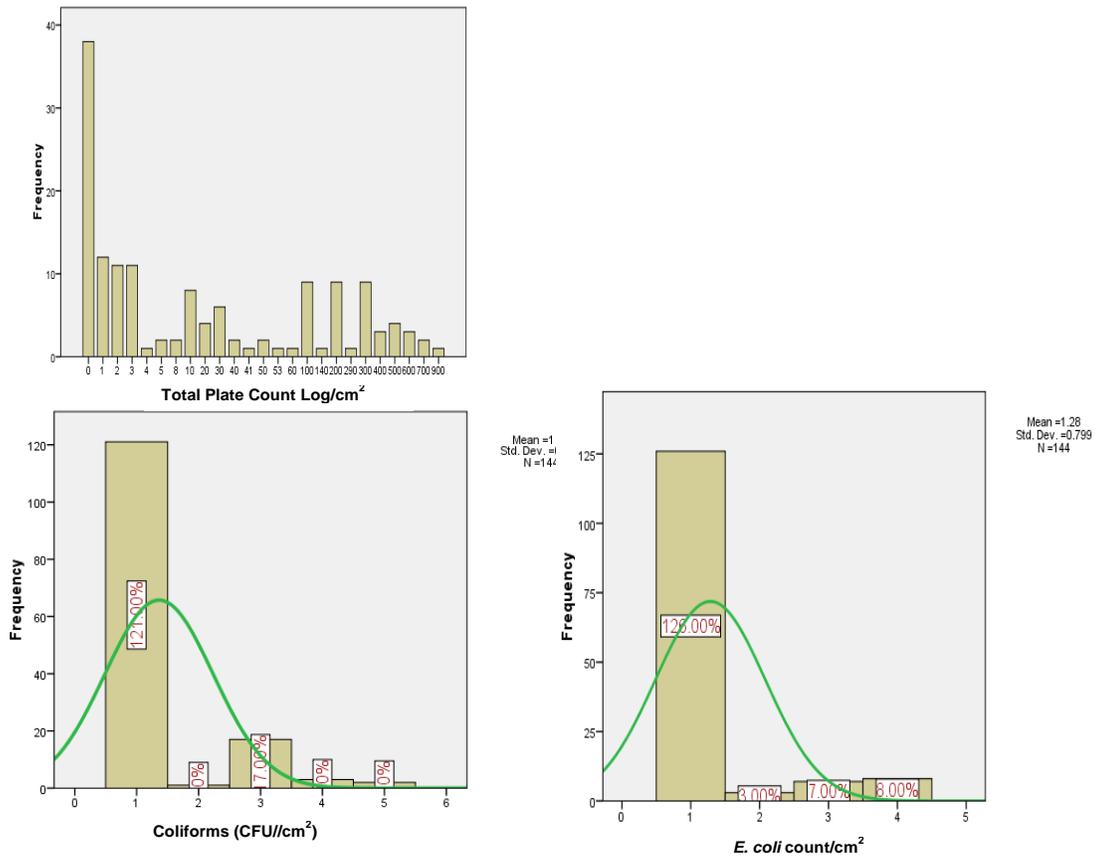


Figure 4. The microbial category distribution safety were acceptable based on international standards in cumulative 91% TPC, 96.5% CFU and 94.4% *E. coli*. a, TPC log/cm² distribution category in the carcass, from 0=no detection (46.5% excellent) to 900 (9% marginal). b. CFU/cm² from 84% excellent for no detection to 1.39% unacceptable. c. *E. coli*/cm² from 87.5% excellent for no detection to 5.56% marginal.

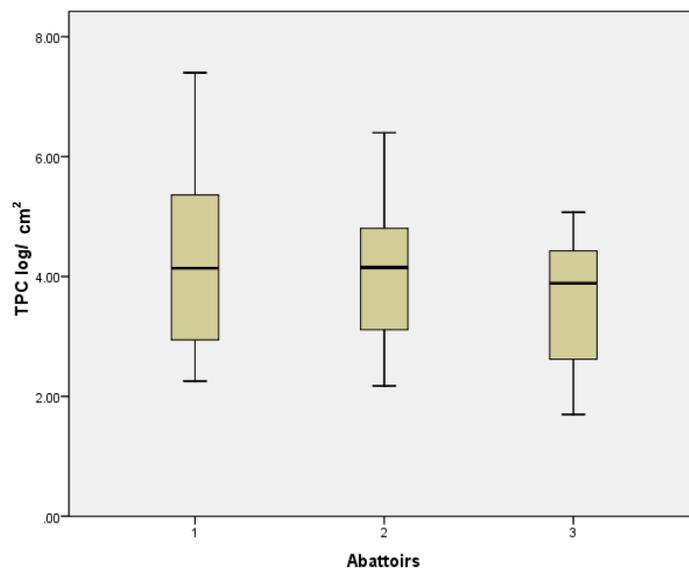


Figure 5. TPC log/cm² distribution in abattoirs 1, 2 and 3.

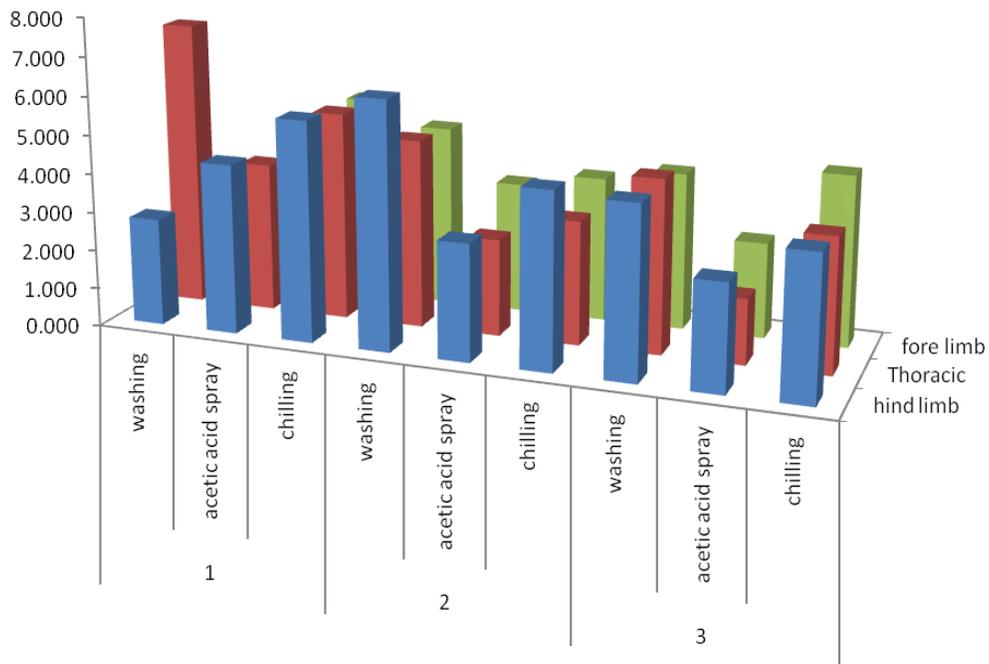


Figure 6. TPC log/cm² in Abattoirs 1, 2 and 3 goat carcass parts swapped post washing, acetic acid spray and chilling.

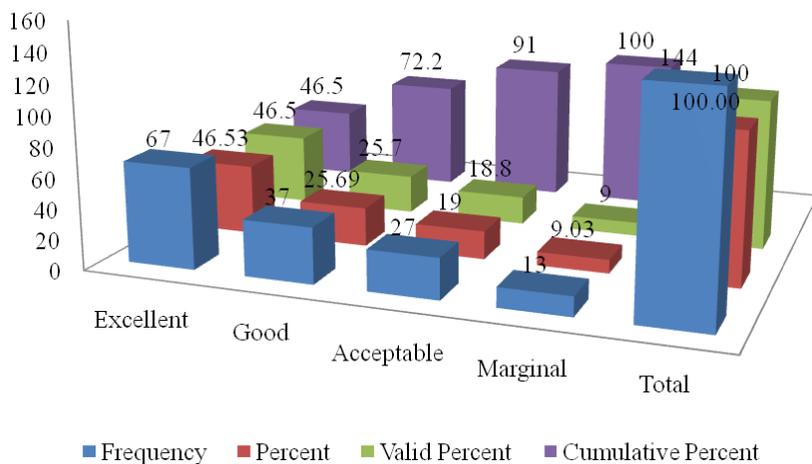


Figure 7. TPC log/cm² carcass safety category distribution.

Abattoirs 1, 2 and 3 performance of TPC frequency indicated that the carcass dressing practices were apt and of international standards (Figure 8).

Figure 8 shows that CFU wise, 96.5% of the carcass was safe cumulatively of which 84% was excellent, 0.7% good, 11.8% acceptable, and 2.1% marginal where action is required. However, apron from clean area in abattoir 1 and acetic acid sprayed and chilled thorax in

abattoir 2 in 1/4 sampling days scored 1.39% unacceptable result that is great time to warn the HACCP team and the abattoirs' top management to strictly implement HACCP procedures for quality meat products routinely.

Figure 9, indicated that *E. coli* wise 94.4% of the carcass was safe cumulatively of which 87.5% was excellent, 2.1% good, 4.9% acceptable and 5.6%

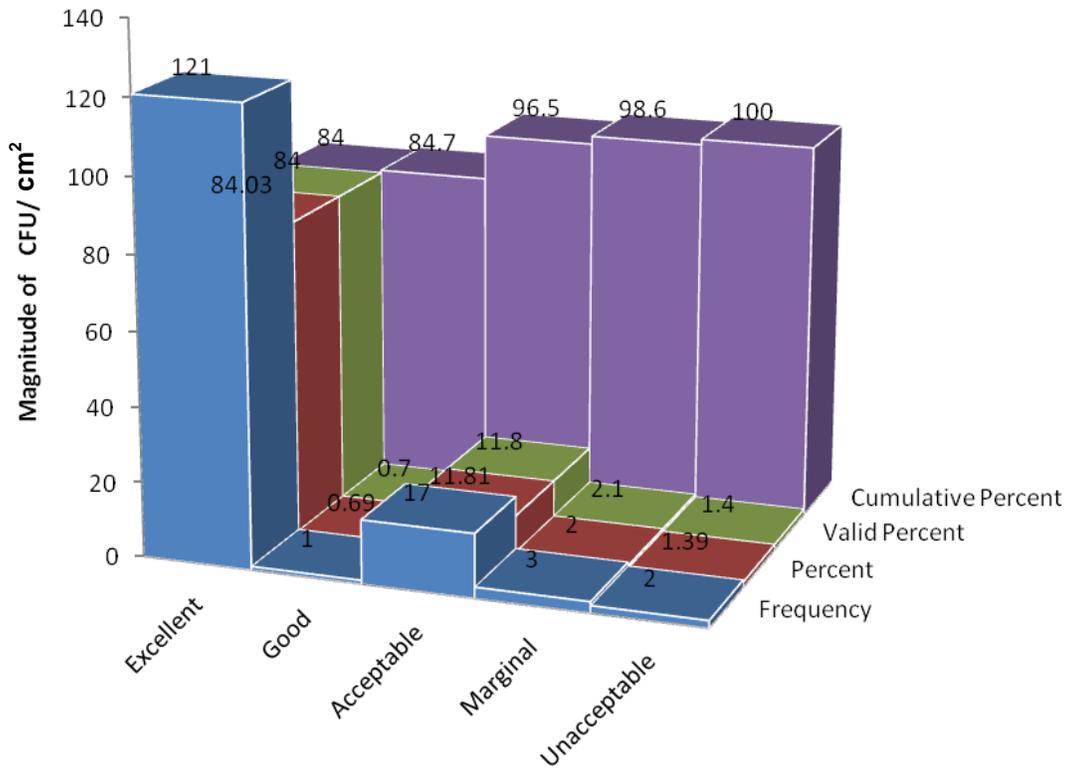


Figure 8. Frequency of CFU/cm² category distribution.

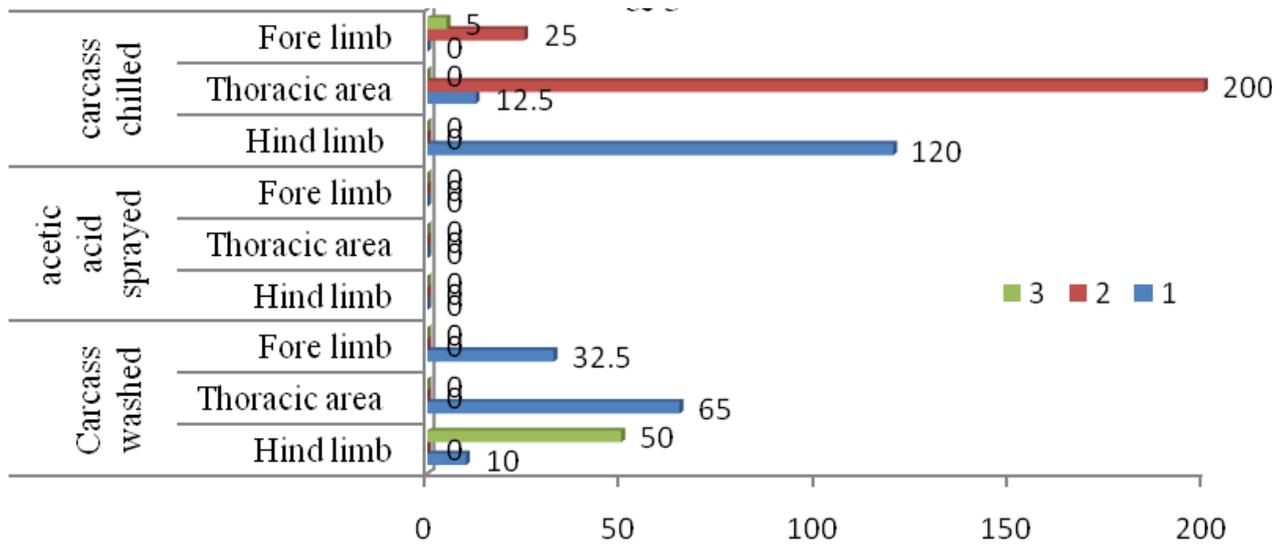


Figure 9. *E. coli* counts/cm² distribution in category in the swapped part of goat carcass in the sequential meat processing.

marginal. Marginal score resulted in acetic acid sprayed and chilled hind limb at the 1st sampling day, and 2nd sampling day, washed fore limb and thorax, and

employee's apron from clean area of abattoir 1; simultaneously employee's palm from clean area of abattoir 2, and in the washed hind limb of abattoir 3 at the

3rd sampling day, that is not acceptable on fresh meat, indicating precautionary measures of meat hygiene along the slaughter and meat handling chains.

E. coli counts/cm² in abattoir 2 scored 200 in the thorax post acetic acid sprayed and chilling followed by abattoir 1 of apron in the clean area that scored 175 *E. coli*/cm². Thorax post acetic acid spray and chilling more contaminated by *E. coli* in abattoir 2 thorax followed by hind limb post washing was more contaminated by TPC log/cm² in abattoir 1.

The rate of carcass contamination was related to the higher contact area by the abattoir employee engaged to move manually goat or beef carcass conveniently by Ahouandjrou et al. (2015). However, Ahouandjrou et al. (2015) in Benin reported that there was 100% unsatisfactory (6.16±0.17) TPC log/cm² contamination in beef thigh while Zweifel and Stephen (2003) reported high contamination in neck and chest sites that contradict with the Ethiopian export abattoirs' goat carcass contamination mean findings of acceptable category (3.9±0.2 TPC log/cm²), ranged 1.7 TPC log/cm² in abattoir 3 at the thoracic area after acetic acid spray to 7.4 TPC log/cm² at thorax after washing. There was no detection of CFU and *E. coli* in the carcass washing water, but significantly higher TPC log/cm², although it was categorically excellent distribution. Comparatively, in abattoir 1, there was higher bacterial contamination since both CFU and *E. coli* were in marginal category in employee apron working in clean area, followed by abattoir 2 where TPC log/cm² was marginal category that demands action for strict correction measures of HACCP implementation.

The bacterial loads decline from washing to acetic acid spray to chilling in the abattoirs in the carcasses' sampled parts, confirming that the sequential procedures do have vital effects in minimizing the bacterial counts, consistent to Bhandare et al. (2007, 2010) and FAO (2007). The research found meat contamination with significantly higher mean ($P < 0.05$) of 1.9±1 TPC/cm² and least in *E. coli*/cm² (1.28±0.8) that agreed with research findings of Haque et al. (2008) goat carcass report. On the basis of microbiological standards of raw meat, the finding of the microbial counts was indeed similar to Australian carcass standards that meat from well-controlled processes will usually be in the excellent or good categories, with only occasional departures into the acceptable category. It would be very unusual for these products to have TPC or *E. coli* count in the marginal category and other count is a trigger for investigating reasons for high counts, and for Corrective Action (AS4696-2002), consistent to this research findings of *E. coli* wise, 94.4% of the carcass was safe in cumulative of which 87.5% was excellent, 2.1% good, 4.9% acceptable and 5.6% marginal, 91% TPC of the carcass was safe in cumulative of which 46.5% was excellent, 25.7% good, 18.8% acceptable and 9% marginal. Coliforms wise, 96.5% of the carcass was

safe in cumulative of which 84% was excellent, 0.7% good, 11.8% acceptable and 2.1% marginal. However, apron from clean area in abattoir 1 and thorax in chiller of abattoir 2 scored 1.39% unacceptable result, which alerts the HACCP team and the abattoirs' top management to strictly implement HACCP procedures for quality meat products routinely. Similar values to those presented in the study were confirmed by Jahan et al. (2015) whose findings stated 40% satisfactory and 32% acceptable for TPC, however, disagreed with 28% rejected resources which is the highest in comparative to our research. Martinez et al. (2010) has reported that most of the samples (63.7%) had TPCs of 4.1 to 5.0 CFU log/cm², and most of the carcasses (49.8%) had ECs of 1.1 to 2.5 CFU log/cm². According to the International Standard Organization (ISO 4833: 2003), TPC of 80% of analyzed samples must not exceed 5 log cfu/g, whereas 20% of the samples may have counts of up to 5 log cfu/g.

CFU strongly correlate with *E. coli* and weakly correlate with TPC. CFU significantly (0.01) correlate with *E. coli* and at 0.05 significance level with TPC. So strong correlation in between coliform and *E. coli* could have severe consequence in meat quality and risk in consumers' health. Haque et al. (2008) found a significant correlation between TPC and CFU that disagreed with the present research findings. The reduction of TPC after treatment in this study may be attributed to proper slaughtering procedures resulting in decreased level of contaminating bacteria (Aftab et al., 2012). However, there could be higher bacteria in chilling in abattoir 1 similar to research conducted by Abdalla et al. (2009) whose findings increased post washing. Jeffery et al. (2003) and Abdalla et al. (2009) found 3.74±0.02 log/cm² on workers' hands similar to our study findings of workers' hands and apron along with carcass washing water scored TPC log/cm² 3.93±1.29 that was also supported by Jeffery et al. (2003) who confirmed workers' hands and equipments as sources of meat contaminations.

CONCLUSION AND RECOMMENDATION

The study was conducted to investigate the targeted organisms TPC, CFU and *E. coli* and were with safely acceptable range, but coliform result was unacceptable (1.39%) in abattoir 1 in employee apron in the 2nd visit day and abattoir 2 in the thorax post chilling in the 2nd visiting day that alerts the HACCP team although 96.5% of the carcass was safe cumulative wise of which 84% was excellent. The result confirmed that the bacterial loads decline from washing to acetic acid spray to chilling in three of the abattoirs' sampled carcass parts, confirming that the sequential procedures do have vital effects in minimizing the bacterial counts. Employee apron and thorax part of the carcass displayed bacterial

contamination to alert the abattoirs management. Carcass contaminating bacteria should be determined. Besides to the refreshing training need of abattoir employees, the abattoir supervisors could be source of contamination for they used to wander in and out restlessly with negligence of clean to dirty area routine procedures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful for the collaboration received from the management of the abattoirs where samples were collected, Ethiopian Meat and Dairy Industry Development Institute for providing the required facilities to carry out this research work, and laboratory technician Selam Tariku for her supports of sampling.

REFERENCES

- Abdalla MA, Suliman SE, Ahmed DE, Bakhie AO. (2009). Estimation of bacterial contamination of indigenous bovine carcasses in Khartoum (Sudan). *African Journal of Microbiology Research* 3(12):882-886.
- Aftab M, Rahman A, Qureshi M, Akhter S, Sadique U, Sajid A, Zaman S (2012). Level of Salmonella in Beef of Slaughtered Cattle at Peshawar. *The Journal of Animal and Plant Sciences* 22(2):24-27.
- Ahouandjrou H, Baba-Moussa F, Bonnou J, Dougnon V, Adeoti Z, Yedji R, Toukourou F, Baba-Moussa L (2015). Evaluation of the Microbiological quality of cattle carcasses in some slaughterhouses at Benin, West Africa. *International Journal of Scientific Report* www.sci.rep.com
- AS4696 (2002). Microbiological testing for process monitoring in the meat industry guidelines as of Australian standard for hygienic production and transportation of meat and meat products for human consumption AS4696-2002, section 3.12 requirement.
- Bhandare S, Ashish MP, Vikas SW, Ravindra JZ (2010). Prevalence of microorganisms of hygienic interest in an organized abattoir in Mumbai, India. *Journal of Infection in Developing Countries* 4(7):454-458.
- Bhandare SG, Sherikar AT, Paturkar AM, Waskar VS, Zende RJ (2007). A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food Control* 18(7):854-858.
- European Commission (EC) (2007). EC Regulation No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union* L 322/15.
- Food and Agriculture Organization (FAO) (2007). Food and Agriculture Organization: Statistical database: 2007. Available: <http://faostat.fao.org/site/339/default.aspx>.
- Haque M, Siddique P, Habib MA, Sarkar V, Choudhury KA (2008). Evaluation of sanitary quality of goat meat obtained from slaughter yards and meat stalls at late market hours. *Bangladesh Agricultural University, Bangladesh Journal of Veterinary Medicine* 6(1):87-92
- Hamdan MH, Mohamed IMF, Siham ES, Ismail MF, Mohammed ASA (2019). Microbiological Quality of Goat Carcasses at different stages of slaughter in Dallam Island Abattoir. *International Journal of Current Microbiology and Applied Sciences* 8(3):242-250.
- IBM Corporation (2011). SPSS Statistics version 20.
- ISO 4833 (2003). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30°C.
- Jahan F, Mahbub E-E, Siddique A (2015). Bacteriological Quality Assessment of Raw Beef Sold in Sylhet Sadar. *Sylhet Agricultural University, Bangladesh. A Scientific Journal of Krishi Foundation: The Agriculturists* 13(2):9-16.
- Jeffery B, Donald B, Gill O (2003). Implementation of validated HACCP system for the control of microbiological contamination of pig carcass at a small abattoir. *Canadian Veterinary Journal* 44(1):51-55.
- Magnus P (1981). Meat composition. Food Science and Technology 14th Ed. Gobumunary Pub. London pp. 108-215.
- Martinez B, Celda M, Begon A, Ines G, Lopez-Mendoza M (2010). Microbiological Sampling of Carcasses by Excision or Swabbing with Three Types of Sponge or Gauze. *Journal of Food Protection* 73(1):81-87.
- Mayr D, Margesin R, Klingsbichel E, Hartungen ED, Jenewein D, Schinner F, Mark TD (2003). Rapid detection of meat spoilage by measuring volatile organic compounds by using proton transfer reaction mass spectrometry. *Applied and Environmental Microbiology* 69:4697-4705.
- Mead GC (2007). Microbiological analysis of red meat, poultry and eggs. Wood head Publishing Limited and CRC Press NY, USA. pp 83-97 and 148-160.
- Nagarajan V, Wahab A, Shivraj S (2018). Study of bacterial contamination of raw meat in Hyderabad. *MOJ Proteomics & Bioinformatics* 7(1):46-51.
- Nega T (2015). SPS & Related requirements of importing countries in middle East, North and West Africa and South East Asia. *Societa cooperativa a responsabilita limitata (OPERA-Italy) Addis Ababa-Ethiopia*.
- Nouchi S (2009). Superficial Bacterial Contamination of Ovine and Bovine Carcasses at El-Harrach Slaughterhouse. *Algeria. European Journal of Scientific Research* 38(3):474-85.
- Sofos JN (2008). Challenges to meat safety in the 21st century. *Meat Science* 78(1-2):3-13.
- Verhille S (2013). Understanding microbial indicators for drinking water assessment: interpretation of test results and public health significance. *National collaborating centre for environmental health* pp. 1-12.
- Wen X, Chen F, Lin Y, Zhu H, Yuan F, Kuang D, Jia Z, Yuan Z (2020). Microbial indicators and their use for monitoring drinking water quality. *A Review Sustainability* 12(6):2249.
- Zweifel C, Stephan R (2003). Microbiological monitoring of sheep carcass contamination in three Swiss abattoirs. *Journal of Food Protection* 66(6):946-952.

Related Journals:

