OPEN ACCESS

African Journal of Microbiology Research

June 2022
ISSN 1996-0808
DOI: 10.5897/AJMR
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

About AJMR

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

Indexing

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

Open Access Policy

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

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

Article License

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

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

Article Copyright

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

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

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

Self-Archiving Policy

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

Digital Archiving Policy

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

Metadata Harvesting

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

Memberships and Standards

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

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

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

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

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

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

Contact

Editorial Office: ajmr@academicjournals.org

Help Desk: helpdesk@academicjournals.org

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

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

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

Editors

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

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

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

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

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

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

Dr. Franco Mutinelli
Istituto Zooprofilattico Sperimentale delle
Venezie Italy.

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

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

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

Prof. Natasha Potgieter
University of Venda
South Africa.

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

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

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

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

Editors

Dr. Afework Kassu
University of Gondar
Ethiopia.

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

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

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

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

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

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

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

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

Dr. Samuel K Ameyaw
Civista Medical Center
USA.

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

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

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

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

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

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

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

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

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

Table of Content

Antibiotic susceptibility of bacteria isolates from ward environment of a hospital in Tema, Ghana 211
John Antwi Apenteng, Esther Eyram Asare Yeboah and Gertrude Kyere-Davies

Antibiogram and multidrug resistant pattern of Escherichia coli from environmental sources in Port Harcourt 217
Agbagwa O. E., Chinwi C. M. and Horsfall S. J.

Aflatoxins B1 contamination levels in maize and awareness of aflatoxins among main maize stakeholders in Chemba and Kondoa Districts, Tanzania 223
Asha Hamad Ndwata, Suleiman A. Rashid, Davis Noboth Chaula

Assessment of handwashing knowledge, attitude and practices among healthcare workers at Muhimbili National Hospital, Tanzania 238
Deus M. Mtweve and Raphael Z. Sangeda

Biodegradability of polystyrene plastics by bacterial isolates from plastic composted waste soil and molecular characterization of plastic degrading bacterial isolates 247
Ugueri Udochukwu, E. I. Atuanya and Zainab Usman

Vol. 16(6), pp. 211-216, June 2022
DOI: 10.5897/AJMR2020.9338
Article Number: 4171E1469173
ISSN: 1996-0808
Copyright©2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Antibiotic susceptibility of bacteria isolates from ward environment of a hospital in Tema, Ghana

John Antwi Apenteng[1]*, Esther Eyram Asare Yeboah[1] and Gertrude Kyere-Davies[2]

[1]Department of Pharmaceutical Science, School of Pharmacy, Central University, Miotso, Ghana.
[2]University of Oklahoma Health Science Centre, United States.

Received 14 April, 2020; Accepted 23 March, 2022

Community and hospital-acquired antimicrobial resistance is on the increase worldwide and threatens the ability to treat patients effectively. This can result in high levels of morbidity and mortality from microbial infections. Susceptibility patterns help track microbial resistance potentials in order to enhance antibiotic prescription and use. The susceptibility patterns of *Staphylococcus aureus* and *Salmonella typhi* from the wards of a major hospital in the Tema Metropolis of the Greater Accra region of Ghana were studied. Fifty-seven *S. aureus* and 12 *S. typhi* isolates were confirmed from 150 samples collected from the various parts of the hospital wards. The isolates were evaluated for their susceptibility/resistance against five antibiotics namely: Cefuroxime, gentamicin, tetracycline, ciprofloxacin, and erythromycin using the Kirby-Bauer disc diffusion method. Results revealed that hospital door handles had the highest number of microbes as compared to other sites. Of the *S. typhi* isolates, 66.67% were resistant to cefuroxime but completely susceptible to gentamicin. Also, 75.44% of *S. aureus* isolates were resistant to cefuroxime but highly susceptible to ciprofloxacin, gentamicin and tetracycline. The results indicate that *S. aureus* and *S. typhi* are gradually developing resistance to cefuroxime which is currently a major antibiotic in the health delivery system of Ghana.

Key words: Susceptibility pattern, antibiograms, *Staphylococcus aureus*, *Salmonella typhi*, hospital wards.

INTRODUCTION

One of the major problems in human health is the emergence and spread of antibiotic resistance which has resulted in the limited success of antibiotics in the treatment and prevention of infectious diseases (Dagnachew et al., 2014). Although antimicrobial resistance is a problem to disease pathology, one of its major outcomes is the problem of limited therapeutic options (Chatterjee et al., 2016). Community and hospital-acquired antimicrobial-resistant strains of bacteria especially Gram-negative bacteria such as

*Corresponding author. E-mail: jApenteng@central.edu.gh/j.a.apenteng@gmail.com. Tel: +233 249 449 249 / +233 547 165 573.

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

Klebsiella pneumonia are on the increase worldwide (Caneiras et al., 2019). This problem, threatens the effective treatment of patients and therefore results in the need for emphasizing new treatment alternatives, infection control, continuous surveillance and appropriate antimicrobial prescription (WHO, 2020).

In Ghana, *Salmonella typhi* infection is ranked amongst the top 20 causes of outpatient morbidity and 1.2% of all hospital admissions (Fusheini and Gyawu, 2020). *Staphylococcus aureus* is also one of the most common causes of infections reported in hospitals in Ghana. Although a common pathogen of economic importance, there are very limited surveillance data on the pathogen in Ghana (Donkor et al., 2018).

Resistance of bacterial pathogens to antibiotics has seen an increase in prevalence and spread over the years largely due to inappropriate use of antibiotics both in health facilities and within the community (Yevutsey et al., 2017). Antimicrobial resistance is a major public health concern in Ghana as it has increasingly become difficult to therapeutically manage infections caused by resistant strains of bacteria and thus could spread rapidly within the population into an epidemic (Yevutsey et al., 2017). Several types of research have indicated a high prevalence of resistance to some commonly used antibiotics such as tetracycline, ampicillin, chloramphenicol, and co-trimoxazole (Asante et al., 2017). Penicillin which was hitherto commonly used is gradually losing its effectiveness against *Streptococcus pneumoniae* and *Neisseria meningitidis* in Ghana (Dayie et al., 2013; Duplesis et al., 2016).

Though antibiotic therapy is highly utilized in Ghana, there is the lack of information on the resistance and susceptibility of bacterial pathogens to antibiotics due to the lack of surveillance in the various healthcare facilities (Labi et al., 2018). Hospital environments and high-touch surfaces could be contaminated with microorganisms (Casini et al., 2019) and could result in their spread to healthcare personnel and patients if not properly disinfected. This problem could result in ineffective antimicrobial therapy (Yevutsey et al., 2017). The Tema Metropolis is located in the Southeastern coast of Ghana. It is one of the two cities in the Greater Accra region of Ghana. The population of Tema Metropolis, according to the 2010 Population and Housing Census, is 292,773 representing 7.3% of the region's total population. It has several private and well-equipped public health facilities (Ghana Statistical Service Report, 2010).

This study was aimed at determining the prevalence and antibiotic profiles of *S. typhi* and *S. aureus* isolated from ward environment of a hospital in the Tema Metropolis of the Greater Accra region of Ghana. *S. aureus* and *S. typhi* are important bacteria in the health delivery system of Ghana. They are mostly transferred through food, human to human, and can cause nosocomial infections (Adzitey et al., 2017; Fusheini and Gyawu, 2020). Hospital personnel, patients, and visitors are all prone to being exposed to these microorganisms due to their presence in the hospital environment.

MATERIALS AND METHODS

Collection of samples at the hospital wards

A swab of sample sites was taken using a sterile cotton bud dipped into sterile distilled water. Samples were taken from the beds, tables, doors, and the floors of various wards, (male, children, and female wards) of the hospital. A total of 150 samples were collected and coded appropriately, placed in a sterile swab bag, and transported immediately to the Central University Microbiology Laboratory. Collected samples were enriched in peptone broth and incubated at a temperature of 37°C for 48 h (Agoba et al., 2017) with slight modifications.

Isolation of *S. aureus* and *S. typhi*

S. aureus

The isolation of *S. aureus* was done by taking a loopful of the enrichment culture and streaking onto the surface of prepared mannitol salt agar. This was incubated at 37°C for 24 h. Pathogenic *S. aureus* were identified as bright-yellow colonies. The colonies were sub cultured on nutrient agar and incubated again at 37°C for further tests. Further biochemical tests namely; gram staining, MRVP, catalase, citrate utilization test and Gelatin hydrolysis test were used in identifying and characterizing the isolates. All microbial culture media used were purchased from Oxoid, UK.

S. typhi

Isolation of *S. typhi* was done according to the method described by Hassan et al. (2016) and the protocol of the WHO global foodborne infection network manual 2016 with slight modifications. A loopful sample of the pre-enriched isolates was streaked onto Bismuth Sulphite agar (BSA), Salmonella-Shigella agar (SSA), and Xylose Lysine Deoxycholate agar (XLD) incubated at 37°C for 18 to 24 h. Shiny black rabbit-eyed colonies on BSA, transparent black-centered red colonies on XLD agar and colourless black-centered colonies on SSA were observed and suspected to be *Salmonella* spp. The colonies were isolated onto nutrient agar and further incubated at 37°C for 24 h. Confirmation of species was done via biochemical tests which include; inoculation and incubation on TSI agar, Motility -Indol -Tests, Citrate utilization test. O and H -antigen serotyping was also conducted. All microbial culture media used were purchased from Oxoid, UK.

Determination of the antibiotic susceptibility profile of the bacterial isolates

The isolates obtained were tested against five antibiotics with specific concentrations namely; ciprofloxacin (CIP) 5 µg, erythromycin (E) 15 µg, cefuroxime (CXM) 30 µg, gentamicin (CN) 10 µg, tetracycline (TE) 30 µg. The bacterial isolate in the Muller-Hinton broth diluted with sterile distilled water to 0.5 MacFarland was inoculated aseptically onto prepared Muller- Hinton agar plates with the aid of sterile cotton bud. The antimicrobial discs (Oxoid, UK), with the aid of the multidisc dispenser, were then placed on the inoculated Muller- Hinton agar plates. The agar plates were then incubated at 37°C for 24 h. After 24 h of incubation, the zones of microbial growth inhibition were measured in millimeters with a meter rule, recorded and interpreted according to the Clinical and

Table 1. CLSI guidelines 2014.

Antibiotic	Content (µg)	Organism	Measures in millimetre (mm)		
			Susceptible (S)	Intermediately susceptible (I)	Resistant (R)
Cefuroxime	30	*S. typhi*	≥23	15-22	14
		S. aureus	≥18	15-17	14
Ciprofloxacin	5	*S. typhi*	≥31	21-30	20
		S. aureus	≥21	16-20	15
Erythromycin	15	*S. typhi*			
		S. aureus	≥23	14-22	13
Gentamicin	10	*S. typhi*	≥15	13-14	12
		S. aureus	≥15	13-14	12
Tetracycline	30	*S. typhi*	≥15	12-14	11
		S. aureus	≥19	15-18	14

Table 2. Prevalence of *S. aureus* and *S. typhi* in wards.

Ward	Number of samples (N)	Number of *S. aureus* isolates n (%)	Number of *S. typhi* isolates n (%)
Male	54	20(37.0%)	5(9.30%)
Female	48	19(39.6%)	5(10.4%)
Children	48	18(37.5%)	2(4.2%)
Total	150	57(38.0%)	12(8.0%)

Laboratory Standard Institute (CLSI) guideline 2014 (Table 1).

RESULTS

Prevalence of *S. aureus* and *S. typhi* at wards

Out of the total of 150 samples collected from the various wards, 57 *S. aureus* isolates were obtained representing 38% of all isolates whiles only 12 of the samples were confirmed as *S. typhi* representing 8% of the samples collected as indicated in Table 2.

Prevalence of *S. aureus* and *S. typhi* from sites of collection within wards

Out of the 57 *S. aureus* isolates obtained, it was observed that 48.7% of the isolates were from the door handles of the various wards which were very high compared to other sites of collection. Also, 11.1% of the *S. typhi* isolates were obtained from the surfaces of the tables in the various wards. Figure 1 gives details from other collection sites.

Antibiotic susceptibility profile of *S. aureus* and *S. typhi*

The *S. aureus* isolates obtained were observed to be highly sensitive to gentamicin (91.23%), ciprofloxacin (100%), and tetracycline (100%). High resistance was observed with cefuroxime (75.44%). The antibiotic susceptibility profile of the *S. typhi* isolates obtained revealed high sensitivity to ciprofloxacin (91.66%), tetracycline (75%) and gentamicin (100%) but high resistance to cefuroxime (66.67%) as indicated in Table 3.

DISCUSSION

The results from the study indicated that the majority of the isolates obtained were *S. aureus* than *S. typhi*. This could be due to the fact that *S. aureus* is a common microbe that is part of the normal microflora of the skin

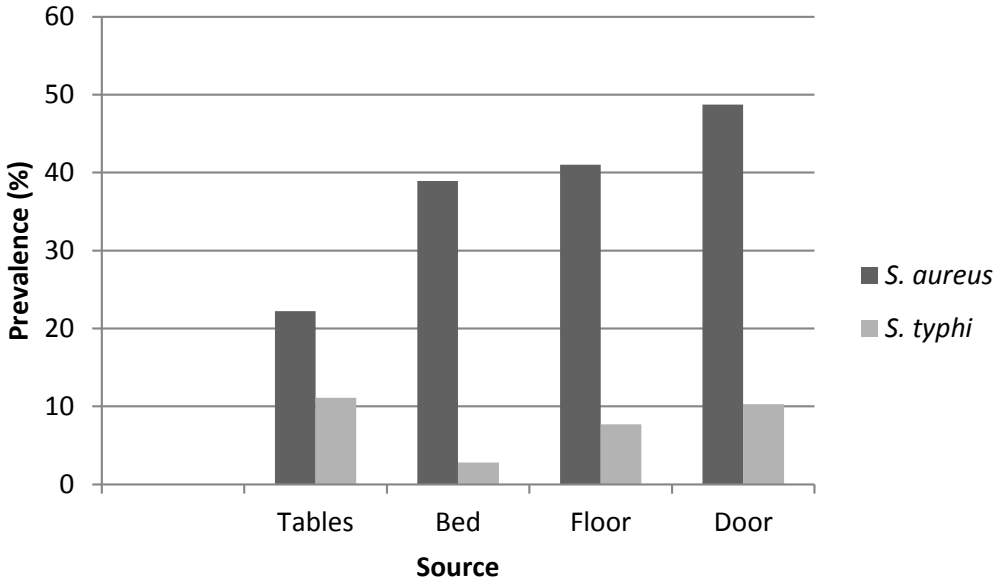

Figure 1. Prevalence of *S. aureus* and *S. typhi* isolates from various sites.

Table 3. Antibiotic susceptibility profile of *S. typhi* and *S. aureus.*

Antibiotic	*S. aureus* n(%)			*S. typhi* n(%)		
	S	I	R	S	I	R
Ciprofloxacin	57(100)	0	0	11(91.66)	0.00	1(8.33%)
Gentamicin	52(91.23)	0	5(8.77)	12(100)	0	0
Tetracycline	57(100)	0	0	9(75)	2(16.67)	1(8.33)
Cefuroxime	5(8.77)	9(15.79)	43(75.44)	3(25)	1(8.33)	8(66.67)
Erythromycin	-	-	-	6(50)	2(16.67)	4(33.33)

S- susceptible, I- Intermediate, R- resistant.

and mucous membranes unlike *S. typhi* which is mostly found in the gut (Tong et al., 2015). Furthermore, *S. aureus* was more prevalent in the female ward than in all the other wards. This is similar to the findings of Dilnessa and Bitew (2016), who reported that *S. aureus* strains are higher in female wards than in male wards revealing a percentage of 53% in females versus 47% in males. The door handles of the hospital had the highest prevalence of microbes as indicated in this study. Studies conducted by Odigie et al. (2017) have also confirmed a high level of microbial contaminations on door handles and this is no exception. The major concern, in this case, is the possibility and frequency of transfer of resistant strains amongst hospital staff, patients, and visitors.

All the *S. aureus* isolates obtained in this study showed some level of resistance to ciprofloxacin, tetracycline, erythromycin and cefuroxime with the exception of gentamicin. This is similar to a study conducted by Onwubiko and Sadiq (2011) in Nigeria where almost all the *S. aureus* isolates (92.4%) were susceptible to gentamicin and had a similar resistance rate to erythromycin (35.8%) (Figure 2). Complete susceptibility to gentamicin could largely be due to the fact that gentamicin is only used by the parenteral route and therefore is not widely abused and not readily available unlike the oral antibiotics. This makes it less exposed to the bacterial pathogens hence the development of resistance is slowed. Furthermore, gentamicin is an aminoglycoside and hence produces unwanted side effects such as ototoxicity which also limits its use by physicians, especially in children.

All the *S. typhi* isolates obtained in this study showed high susceptibility to ciprofloxacin, gentamicin, and tetracycline. There was however, high resistance to cefuroxime (66.77%). Ciprofloxacin has also been found to be a highly effective therapy for infections due to multi-drug resistant *S. typhi* as well as *Neisseria gonorrhoeae* in some countries (Melendez et al., 2019). A similar result was again obtained in a study in Bangladesh where *S. typhi* isolates were highly susceptible to ciprofloxacin

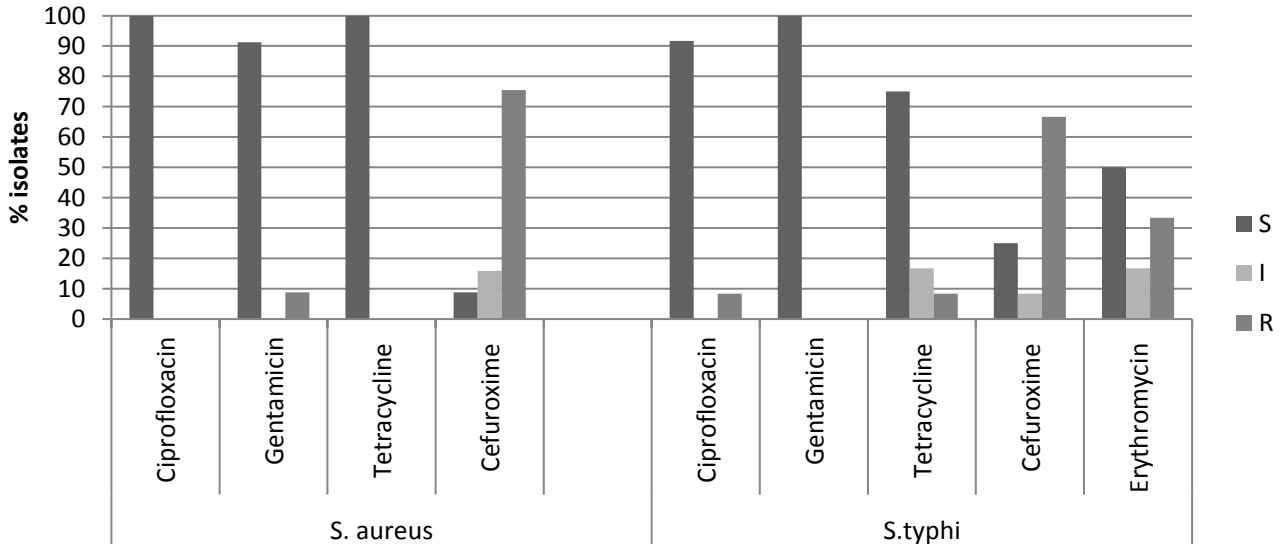

Figure 2. Antibiotic susceptibility pattern of *S. aureus* and *S. typhi* to antibiotics. S- Sensitive, I- Intermediate, R- Resistant.

(Mannan et al., 2014). Tetracycline on the other hand, to which the bacteria were most sensitive is an old drug and is not been widely used in recent times. Consequently, the bacteria might have developed low resistance to it due to the routine use of newly developed antibiotics which probably eliminates resistance against older antibiotics. There is an urgent need for surveillance on antimicrobial resistance to the commonly used antibiotics to determine their effectiveness and to improve treatment outcomes. Disinfection practices should be heightened to decrease the contamination of the ward's environment with resistant strains of bacteria.

Conclusion

The study has revealed the presence of antibiotic-resistant strains of *S. aureus* and *S. typhi* in various parts of the hospital and the potential of easy transfer to patients and workers. This study therefore, indicates the importance of monitoring the usage of antibiotics in human medicine and also the need to reduce the empirical treatment of infections.

Limitations of study

The research focused on phenotypic characteristics of the isolates without the genetic characteristics. Subsequent work on the isolated microbes will look into some resistance genes of interest.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adzitey F, Ekli F, Abu A, Yildiz F (2019). Prevalence and antibiotic susceptibility of *Staphylococcus aureus* isolated from raw and grilled beef in Nyankpala community in the Northern Region of Ghana, Cogent Food and Agriculture 5(1):1671115.

Agoba EE, Adu F, Agyare C, Boamah VE, Boakye YD (2017). Antibiotic resistance patterns of bacterial isolates from hatcheries and selected fish farms in the Ashanti region of Ghana. Journal of Microbiology and Antimicrobials 9(4):35-46.

Asante KP, Boamah EA, Abdulai MA (2017). Knowledge of antibiotic resistance and antibiotic prescription practices among prescribers in the Brong Ahafo Region of Ghana; a cross-sectional study. BMC Health Services Research 17(1):422. Available at: https://doi.org/10.1186/s12913-017-2365-2.

Caneiras C, Lito L, Melo-Cristino J, Duarte A (2019). Community- and Hospital-Acquired *Klebsiella pneumoniae* Urinary Tract Infections in Portugal: Virulence and Antibiotic Resistance Microorganisms 7(5):138.

Casini B, Tuvo B, Cristina ML, Spagnolo AM, Totaro M, Baggiani A, Privitera GP (2019). Evaluation of an Ultraviolet C (UVC) Light-Emitting Device for Disinfection of High Touch Surfaces in Hospital Critical Areas. International Journal of Environmental Research and Public Health 16(19):3572. Available at: https://doi.org/10.3390/ijerph16193572

Chatterjee M, Anju CP, Biswas L, Kumar VA, Mohan CG, Biswas R (2016). Antibiotic resistance in *Pseudomonas aeruginosa* and alternative therapeutic options. International Journal of Medical Microbiology 1(306):48-58.

Clinical and Laboratory Standards Institute (CLSI) (2014) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI Document M100-S24, Wayne 34(1).

Dagnachew M, Yitayih W, Getachew F, Tesfaye N, Kasaw A, Belete B, Habite T, Feleke M (2014). Bacterial isolates and their antimicrobial susceptibility patterns among patients with pus and or wound discharge at Gondar university hospital. BMC Research Notes 7:619. Available at: htpp//dx.doi.org/10.1186/1756-0500-7-619

Dayie NT, Arhin RE, Newman MJ, Dalsgaard A, Bisgaard M, Frimodt-Møller N, Slotved H-C (2013). Penicillin resistance and serotype distribution of *Streptococcus pneumonia* in Ghanaian children less than six years of age. BMC Infectious Disease 13(1):490.

Dilnessa T, Bitew A (2016). Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* with Emphasize on Methicilin Resistance with Patients Postoperative and Wound Infections at Yekatit Hospital

Medical College in Ethiopia. American Journal of Clinical and Experimental Medicine 4(1):7-12.
Donkor ES, Jamrozy D, Mills RO, Dankwah T, Amoo PK, Egyir B, Basoe EV, Twasam J, Bentley SD (2018). A genomic infection control study for Stapylococcus aureus in two Ghanaian hospitals. Infection and Drug Resistance 11:1757-1765.
Duplessis C, Puplampu N, Nyarko E, Carroll J, Dela H, Mensah A, Amponsah A, Sanchez J (2015). Gonorrhea surveillance in Ghana, Africa Military Medicine 180(1):17-22.
Fusheini A, Gyawu SK (2020). Prevalence of Typhoid and Paratyphoid Fever in the Hohoe Municipality of the Volta Region, Ghana: A Five-Year Retrospective Trend Analysis. Annals of Global Health 86(1):1-10.
Ghana Statistical Service (2010). 2010 Population & Housing Census Available at: https://statsghana.gov.gh/gssmain/fileUpload/pressrelease/2010_PHC_National_Analytical_Report.pdf
Hassan AHA, Salam HSH, Abdel-Latef GK (2016). Serological Identification and antimicrobial resistance of Salmonella isolates from broiler carcasses and human stools in Beni-Seuf Egypt. Beni-Seuf University Journal of Basic and Applied Sciences 5(2):202-207
Labi AK, Obeng-Nkrumah N, Nartey ET, Bjerrum S, Adu-Aryee NA, Ofori-Adjei YA, Yawson AE, Newman MJ (2018). Antibiotic use in a tertiary healthcare facility in Ghana: a point prevalence survey. Antimicrobial Resistance and Infection Control 7(1):15.
Mannan A, Shohel M, Rajia S, Mahmud NU, Kabir S, Hasan I (2014). A cross sectional study on antibiotic resistance pattern of *Salmonella typhi* clinical isolates from Bangladesh. Asian Pacific Journal of Tropical Biomedicine 4(4):306-311.
Melendez JH, Hsieh Y-H, Barnes M, Hardick J, Gilliams EA, Gaydos CA (2019). Can Ciprofloxacin be used for Precision Treatment of Gonorrhea in Public STD Clinics? Assessment of Ciprofloxacin Susceptibility and an Opportunity for Point-of-Care Testing Pathogens 8:189.
Odigie AB, Ekhiase FO, Orjiakor PI, Omozuwa S (2017). The Role of Door Handles in the Spread of Microorganisms of Public Health Consequences in University of Benin Teaching Hospital (UBTH), Benin City, Edo State. Pharmaceutical Science and Technology 2(2):15-21.
Onwubiko NE, Sadiq NM (2011). Antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. Pan African Medical Journal 8:4.
Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr (2015). *Staphylococcus aureus* infections: Epidemiology, Pathophysilogy, Clinical Manifestations, and Management. Clinical Microbiology Reviews 28(3):603-61.
WHO Global Foodborne Infections Network (2020). Laboratory Protocal- Isolation of *Salmonella spp* from food and animal faeces. June, 2010. Available at: https://antimicrobialresistance.dk
Yevutsey SK, Buabeng KO, Aikins M, Anto BP, Biritwum RB, Frimodt-Møller N, Gyansa-Lutterodt M (2017). Situational analysis of antibiotic use and resistance in Ghana: policy and regulation. BMC Public Health 17:896.

Vol. 16(6), pp. 217-222, June 2022
DOI: 10.5897/AJMR2022.9633
Article Number: 190C43369194
ISSN: 1996-0808
Copyright©2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Antibiogram and multidrug resistant pattern of *Escherichia coli* from environmental sources in Port Harcourt

Agbagwa O. E.*, Chinwi C. M. and Horsfall S. J.

Department of Microbiology, University of Port Harcourt P. M. B. 5323 East-West Road Choba, Rivers State, Nigeria.

Received 18 March, 2022; Accepted 23 May, 2022

Antibiotics are the most successful form of therapeutics developed for the treatment of disease caused by bacteria. The study aimed to assess the prevalence of *Escherichia coli* and multidrug resistant pattern from environmental sources in Port Harcourt, Rivers State, Nigeria. Forty samples were collected from environmental sources including poultry litter, soil, waste water and cloaca. All samples were inoculated onto prepared Eosin Methylene blue plates and incubated for 24 h at 37°C. Colonies were sub cultured onto sterile nutrient agar plates. Pure isolates were identified using standard microbiological methods. Antibiotic susceptibility was carried out on identified *E. coli.* The study showed that from the samples poultry had 15 (37.5%) *E. coli*, soil 11 (27.5%), waste water 9 (22.5%) and cloaca 5 (12.5%) *E. coli*. However, the highest number of *E-coli* was observed in poultry source and least in cloaca. The results also revealed that the number of *E. coli* from poultry were 7 (46.7%), 5 (33.3%), 2 (13.3%) and 1 (6.7%), soil 6 (54.5%), 1 (9.1%), 3 (27.3%) and 1 (9.1%), waste water 2 (22.2%), 2 (22.2%), 2 (22.2%), 2 (22.2%) and 1 (11.1%) and cloaca 2 (40.0%) and 3 (60.0%), respectively. *E.-coli* were susceptible and resistant to classes of antibiotic including Ceftazidime, Cefuroxime, Gentamicin, Cefxime, Ofloxacin, Augmentin, Nitrofurantoin and Ciprofloxacin. Hence, the study s amongst others that to prevent further emergence and spread of resistant strains in *E-coli*, rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are essential and mandatory

Key words: Antibiogram, *Escherichia coli*, environment, multidrug, resistance.

INTRODUCTION

Escherichia coli are Gram negative pathogen with a global distribution rate. It can be isolated from environmental, clinical, and animal sources. Certain strains of *E. coli* cause most clinical and environmental

*Corresponding author. E-mail: Obakpororo.agabagwa@uniport.edu.ng/ejiroagbagwa@yahoo.com.

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

mediated diseases. Antibiotic resistance has become a worldwide concern due to the emergence of antibiotic-resistant bacteria which limits the clinical use of antibiotics. Antibiotic resistance increases the prevalence of resistant bacteria in both clinical and environmental sources thus rendering available antibiotics ineffective for therapeutic purposes (Ajuga et al., 2021; Odonkor and Kenned, 2018; Agbagwa and Jirigwa, 2015). Sommer et al. (2017) reported that antibiotic-resistant genes responsible for resistance to a wide variety of antibiotics have been identified in a large range of environments including drinking water, waste water, soil and cloaca, etc., in both developed and developing countries. The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens. The potential of the environment to transport microbial pathogens to a greater number of people, causing subsequent illness, is well documented in countries at all levels of economic development. Furthermore, the availability of safe environment is an indispensable feature for preventing epidemic disease and improving the quality of life. Hence, the World Health Organization reported that 80% of all diseases are attributed to unsafe environment. This is to say that developing countries in particular, are plagued with water-related diseases such as diarrhoea which account for 10% of the disease burden in such countries (Ellis and Schoenberger, 2017). Availability of safe environment is a key factor underpinning public health and development of any nation. Environmental sources that may harbour microorganisms include surface water such as lakes, streams, rivers, ponds and underground water such as springs, wells, borehole, soil and animals houses (Oluyege et al., 2009). Lyimo et al. (2016) reported that 748 million, mostly poor and marginalized people still lack access to quality drinking water and safe environment. Of these, almost a quarter (173 million) rely on untreated surface water on a daily basis and over 90% live in rural areas as faecal waste from people and animals is a major source for pollution, particularly in low-income countries.

In 2012, reports had it that approximately 1 billion people in the world did not have access to toilet facilities and instead used open and unsanitary places for defecation especially water bodies. These communities also lack proper water supplies and depend heavily on untreated surface or shallow, unprotected water sources for consumption. Strains of *E. coli* that are pathogenic to both humans and animals are capable of causing disease ranging from self-limiting diarrhoea to life-threatening haemolytic-uremic syndrome and haemorrhagic colitis. However, studies have revealed non-conformity of many water sources in Nigeria to World Health Organization (WHO) standard which has led to faecal contamination of water sources which can extend to other sources (Oluyege et al., 2009). The emergence and dissemination of antimicrobial-resistant (AMR) bacteria is considered the third-largest threat to global public health in the 21st century which reduces the effectiveness of antibiotic treatment and thus leads to increased morbidity, mortality, and healthcare expenditures (WHO, 2014). Hence, the *E. coli* found in people and animals is considered a potential reservoir for AMR genes and these genetic traits can be transferred to or to other bacteria found in people, animals, and in the environment (Katakweba et al., 2018). The study intends to assess the prevalence of *E. coli* and multidrug resistant pattern from environmental sources in Port Harcourt, Rivers State, Nigeria to provide and guide concerted policies for necessary interventions.

MATERIALS AND METHODS

Study area

The research was carried out at the Medical Laboratory of the Department of Microbiology in the University of Port Harcourt which is located at Choba, Rivers State, Nigeria.

Sample collection

Forty samples were collected from environmental sources including poultry, soil, waste water and cloaca. 15 samples were from poultry, soil (11), waste water (9), and cloaca (5). All samples were preserved in cold boxes, transported to the Medical Laboratory of the Department of Microbiology in the University of Port Harcourt within 4 h and maintained at 4°C until use.

Isolation and identification of *E. coli*

All environmental samples (poultry, soil, waste water soil and cloaca) were inoculated on prepared Eosin Methylene Blue (EMB) agar plates and incubated for 24 h at 37°C. The colonies on the plates were sub cultured onto nutrient agar plates (Oxiod) to obtain pure colony. Pure colonies were stored and subjected to Gram staining selected biochemical test such as: citrate test, indole test, oxidase test, triple sugar iron agar test, methyl red and Voges-Proskuer test for identification (Cheesbrough, 2006). They were further confirmed using *E. coli* specific 16s rRNA gene fragment of Ec16 primer pairs (F 5'-GACCTCGGTTAGTTCACAGA-3' and R 5'-CACACGCTGACGCTGACCA-3') (Islam et al., 2016). The reaction mixture was prepared by the addition of 3 µl of *E. coli* DNA, 10 µl PCR master mix, 1 µl of each of the two primers and 6 µl of nuclease free water. The primers have an annealing temperature of 55°C and result in a product with base pair of 588 bp (Islam et al., 2016).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out on identified isolates by the disc diffusion method (CLSI, 2014). In brief, isolates were inoculated on sterile nutrient broth for 16 to 18 h of incubation at 37°C. Inoculum size was adjusted 0.5 McFarland standards and swabbed onto Muller-Hinton agar. Antibiotic disc was placed and plates were incubated for 24 h at 37°C. The zone of inhibition was measured to the nearest millimetre and all bacterial isolates were classified as sensitive, intermediate, and resistant.

Table 1. Multi-drug resistant *E. coli* from environmental sources.

Source	Antibiotic	No. of MDR *E. coli* (n=15)	Percentage of MDR *E. coli*
Poultry	CAZ-CRX-AUG-CXM	7	46.7
	CAZ-CRX-CXM-AUG-GEN	5	33.3
	CAZ-CRX-CXM-AUG-GEN-OFL	2	13.3
	CAZ-CRX-CXM-NIT	1	6.7
		(n=11)	
Soil	CAZ-CRX-AUG-AUG	6	54.5
	CAZ-CRX-CXM-AUG-CPR	1	9.1
	CAZ-CRX-CXM-AUG-NIT	3	27.3
	CAZ-CRX –GEN-CXM-AUG	1	9.1
		(n=9)	
Waste water	CAZ-CRX-GEN-CXM-AUG-NIT	2	22.2
	CAZ-CRX-CXM-AUG-NIT	2	22.2
	CAZ-CRX-CXM-NIT	2	22.2
	CAZ-CRX-CXM-AUG	2	22.2
	CAZ-CRX-AUG	1	11.1
		(n=5)	
Cloaca	CAZ-CRX-CXM-AUG	2	40.0
	CAZ-CRX-GEN-CXM-AUG	3	60.0

CAZ= Ceftazidime, CRX= Cefuroxime, GEN= Gentamicin, CXM=Cefxime, OFL= Ofloxacin, AUG= Augmentin, NIT= Nitrofurantion, and CPR= Ciprofloxacin.
Source: Authors

RESULTS

Of the fifty samples collected from various sources, poultry had 15 (37.5%) *E. coli* samples, soil 11 (27.5%), waste water 9 (22.5%) and cloaca 5 (12.5%) *E.coli*. However, the highest number of *E. coli* was observed in poultry source and least in cloaca sample. Detailed result of the overall prevalence of E.*coli* is presented in Figure 1. Table 1 shows that the number of MDR *E. coli* from poultry was 7 (46.7%), 5 (33.3%), 2 (13.3%) and 1 (6.7%), soil 6 (54.5%), 1 (9.1%), 3 (27.3%) and 1 (9.1%), waste water 2 (22.2%), 2 (22.2%), 2 (22.2%), 2 (22.2%) and 1 (11.1%) and cloaca 2 (40.0%) and 3 (60.0%), respectively. The identified 40 *E. coli* were subjected to antibiotic susceptibility testing. Results obtained showed that *E. coli* from poultry was 47% susceptible, 1% intermediate, and 74% resistant to antibiotic susceptibility test (Figure 2). *E. coli* from soil (Figure 3) was 33% susceptible, 6% intermediate and 49% resistant to the antibiotic tested. *E. coli* from waste water (Figure 4) was 28% susceptible, 3% intermediate, and 41% resistant to antibiotic susceptibility test and Figure 5 shows that *E. coli* from cloaca was 16% susceptible, 1% intermediate, and 23% tested antibiotics.

DISCUSSION

The aim of the study was to assess the prevalence of *E. coli* and multidrug resistant pattern from environmental sources. The finding of the study showed that *E. coli* were isolated from poultry, soil, waste water and cloaca. Fifteen numbers of *E. coli* samples were isolated poultry, soil 11, waste water 9, and cloaca 5. Detailed results are as shown Figure 1 (Overall prevalence of *E. coli*). Isolates were identified by standard microbiological methods. However, colonial morphology for identification is presented. The results showed that from the samples collected from various sources, poultry had 15 (37.5%) *E. coli* samples, soil 11 (27.5%), waste water 9 (22.5%) and cloaca 5 (12.5%) *E. coli*, this shows the presence of multi-drug resistant *E. coli* in the various samples. The finding of this study confirms that of Galindo-Mendez (2020), Singh et al. (2020) in Indian whose studies reported the prevalence of antibiotic resistant genes among multi-drug resistant *E. coli.* However, these studies were sampled in human faeces and at least two antibiotic classes were detected. The finding of this study is in conformity with that of Rubab and Oh (2021), Jahantigh et al. (2020) whose studies discovered the

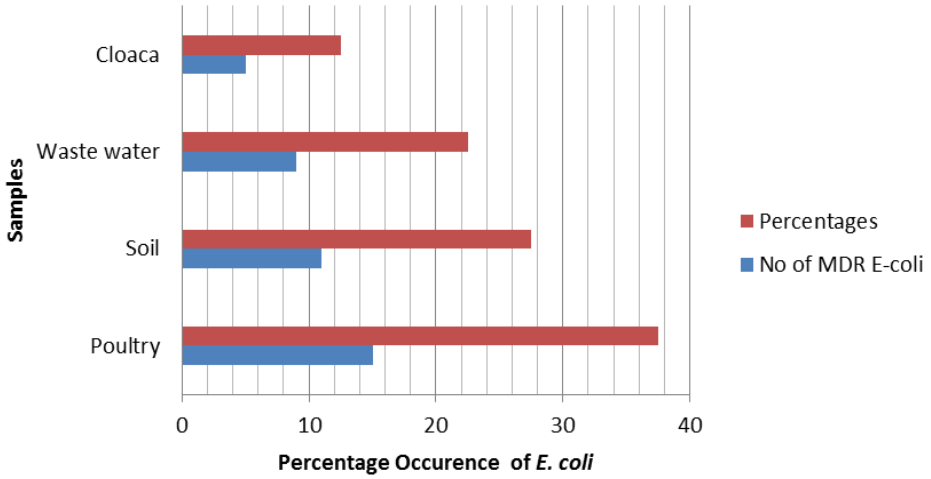

Figure 1. Overall prevalence of *E. coli* form environmental sources.
Source: Authors

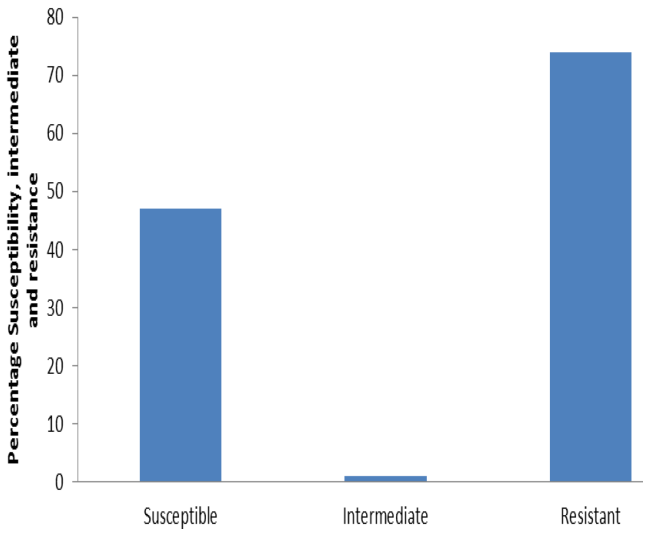

Figure 2. Antibiotic susceptibility of *E. coli* from poultry litter.
Source: Authors

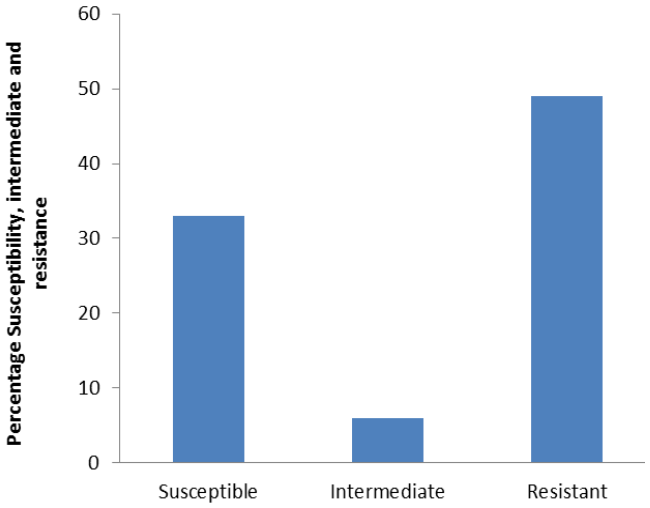

Figure 3. Antibiotic susceptibility of *E. coli* from soil.
Source: Authors

presence of multi-drug resistant in *E. coli*. However, most of these studies were done among STEC isolates and lesions in broiler chickens with gentamicin being the most resistant. By implication, these results indicated that there is high level of the prevalence of multi-drug resistant *E. coli* both in the studied area and other studies as confirmed by Adesoji et al. (2015) and the present study. The present study disagrees with the study carried out by some researchers where the resistant level was higher than the present study. This difference observed could be attributed to the environmental factors, the strain, samples source and other factors (Karami et al., 2006; Xi et al., 2009; Coleman et al., 2012; Chen et al., 2017; Sanganyado and Gwenzi, 2019; Praveenkumarreddy et al., 2020). Multidrug resistant E. coli is currently on the increase and more prevalent in developing countries where antibiotic are used indiscriminately in agriculture, veterinary and medicine. Antibiotics are used in agriculture and animals without proper investigation and policies to guide the use of antibiotics. This can be a major avenue for the transfer of antibiotic resistant bacteria to humans via contaminated environmental

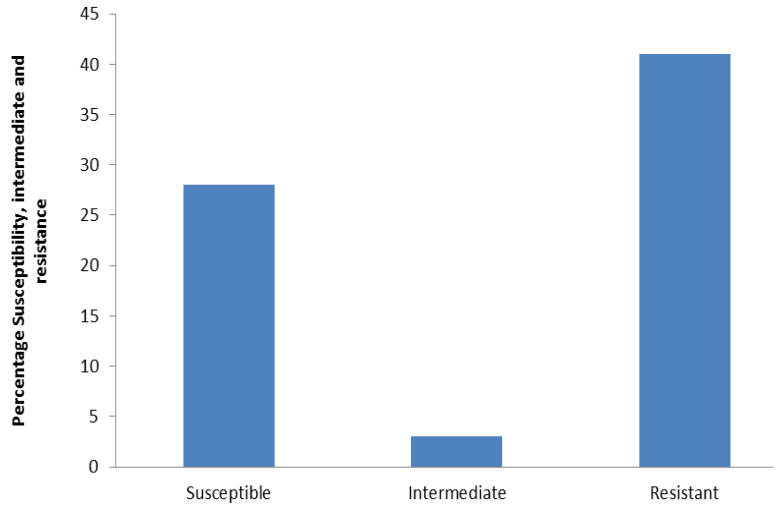

Figure 4. Antibiotic susceptibility of *E. coli* from waste water.
Source: Authors

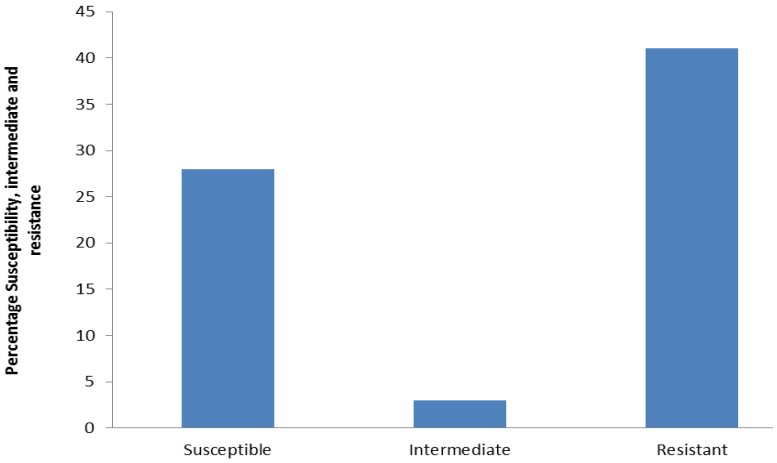

Figure 5. Antibiotic susceptibility of *E. coli* from cloaca.
Source: Authors

sources (Ukah et al., 2018).

Conclusion

This study shows the presence of multi-drug resistant *E. coli* with most showing susceptible and resistance to classes of antibiotic including Ceftazidime, Cefuroxime, Gentamicin, Cefxime, Ofloxacin, Augmentin, Nitrofurantoin and Ciprofloxacin. Hence, to prevent further emergence and spread of MDR resistant *E. coli,* policies guiding the use of antibiotics and regular monitoring of antimicrobial resistance patterns should be put in place to prevent the transfer of resistant bacteria from one source to another.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agbagwa OE, Jirigwa CE (2015). Antibiotics resistance and plasmid

profile of *Staphylococcus aureus* from wound swabs in Port Harcourt Nigeria. Current Research in Bacteriology 8(3):70-76.
Ajuga MU, Otokunefor K, Agbagwa OE (2021). Antibiotic resistance and ESBL production in *Escherichia coli* from various sources in Aba metropolis, Nigeria Bulletin National Research Center 45(1):173.
Adesoji AT, Ogunjobi AA, Olatoye IO, Douglas DR (2015). Prevalence of tetracycline resistance genes among multi-drug resistant bacteria from selected water distribution systems in southwestern Nigeria. Annals of Clinical. Microbiology Antimicrobials 14(1):1-8.
Cheesbrough M (2006). District Laboratory Practice in Tropical countries Part 1&2. Second edition update, Cambridge university press.
Chen Z, Yu D, He S, Ye H, Zhang L, Wen Y, Chen S (2017). Prevalence of antibiotic-resistant *Escherichia coli* in drinking water sources in Hangzhou city. Frontiers Microbiology 8: 1133. Clinical and Laboratory Standards Institute (2014). Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement. Clinical and Laboratory Standard Institute Wayne, Pa. M100- S15, 25:1.
Coleman BL, Salvadori MI, McGeer AJ, Sibley KA, Neumann NF, Bondy SJ, Gutmanis IA, McEwen SA, Lavoie M, Strong D, Johnson I (2012). The role of drinking water in the transmission of antimicrobial-resistant *E. coli*. Epidemiology and Infection 140(4):633-642.
Ellis H, Schoenberger E (2017). On the identification of associations between five world health organization water, sanitation and hygiene phenotypes and six predictors in low and middle-income countries. PloS One 12(1):e0170451.
Galindo-Méndez M (2020). Antimicrobial Resistance in *Escherichia coli*. *E. coli* Infections-Importance of Early Diagnosis and Efficient Treatment pp. 1-20.
Islam MA, Kabir SML, Seel SK (2016). Molecular Detection and Characterization of *Escherichia coli* isolated from Raw milk sold in different markets of Bangladesh. Bangladesh Journal of Veterinary Medicine 14(2):271-275.
Jahantigh MK, Samadi RE, Dizaji, Salari S (2020). Antimicrobial resistance and prevalence of tetracycline resistance genes in *Escherichia coli* isolated from lesions of colibacillosis in broiler chickens in Sistan, Iran. BMC Veterinary Research 16:1-6.
Karami N, Nowrouzian F, Adlerberth I, Wold AE (2006). Tetracycline resistance in *Escherichia coli* and persistence in the infantile colonic microbiota. Antimicrobial Agents and Chemotherapy 50(1):156-161.
Katakweba AA, Muhairwa AP, Lupindu AM, Damborg P, Rosenkrantz JT, Minga UM, Mtambo MM, Olsen JE (2018. First report on a randomized investigation of antimicrobial resistance in fecal indicator bacteria from livestock, poultry, and humans in Tanzania. Microbial Drug Resistance 24(3):260-268.
Lyimo B, Buza J, Subbiah M, Smith W, Call DR (2016). Comparison of antibiotic resistant *Escherichia coli* obtained from drinking water sources in northern Tanzania: a cross-sectional study. BMC Microbiology 16(1):1-10.
Odonkor ST, Kennedy KA (2018). Prevalence of Multidrug-Resistant *Escherichia coli* Isolated from Drinking Water Sources, International Journal of Microbiology ID 7204013.
Oluyege JO, Dada AC, Odeyemi AT (2009). Incidence of multiple antibiotic resistant Gram-negative bacteria isolated from surface and underground water sources in south western region of Nigeria. Water Science and Technology 59(10):1929-1936.
Rubab M, Oh DH (2021). Molecular Detection of Antibiotic Resistance Genes in Shiga Toxin-Producing E. coli Isolated from Different Sources. Antibiotics 10(4):344.
Sanganyado E, Gwenzi W (2019). Antibiotic resistance in drinking water systems: Occurrence, removal, and human health risks. Science of Total Environment 669:785-797.
Singh BR, Singh SV (2020). Metallo-β-Lactamase and Extended-Spectrum-β-Lactamase Production by Serratia Strains. Infection and Drug Resistance 13:1295.
Sommer MO, Munck C, Toft-Kehler R, Andersson DI (2017). Prediction of antibiotic resistance: time for a new preclinical paradigm? Nature Review Microbiology 15(11):689-696.
Ukah UV, Glass M, Avery B, Daignault D, Mulvey MR, Reid-Smith RJ, Parmley EJ, Portt A, Boerlin P, Manges AR (2018). Risk factors for acquisition of multidrug-resistant Escherichia coli and development of community-acquired urinary tract infections. Epidemiology and Infection 146(1):46-57.
World Health Organization (WHO) (2014). "Antimicrobial resistance: global report on surveillance 2014". WHO. Archived from the original on 15 May 2015. Retrieved 9 May 2015.
Xi C, Zhang Y, Marrs, CF Ye W, Simon C, Foxman B, Nriagu J (2009). Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Applied and Environmental Microbiology 75(17):5714-5718.
Praveenkumarreddy Y, Akiba M, Guruge KS, Balakrishna K, Vandana KE, Kumar V (2020). Occurrence of antimicrobial-resistant Escherichia coli in sewage treatment plants of South India. Journal of Water, Sanitation and Hygiene for Development 10(1):48-55.

Vol. 16(6), pp. 223-237, June 2022
DOI: 10.5897/AJMR2022.9637
Article Number: AFA605069202
ISSN: 1996-0808
Copyright©2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Aflatoxins B_1 contamination levels in maize and awareness of aflatoxins among main maize stakeholders in Chemba and Kondoa Districts, Tanzania

Asha Hamad Ndwata[1]*, Suleiman A. Rashid[2], Davis Noboth Chaula[2]

[1]Government Chemist Laboratory Authority, P. O. Box 164, Dar es Salaam, Tanzania.
[2]Sokoine University of Agriculture, School of Engineering, Department of Food Sciences and Agro-processing, P. O. Box 3006, Chuo Kikuu Morogoro, Tanzania.

Received 10 April, 2022; Accepted 24 May, 2022

Maize (*Zea mays*) is the staple food for the majority of people in Tanzania which plays a key role in subsistence and a cash crop among actors of the maize value chain. Environmental factors such as soil contamination by fungi, water stress, warm and humid conditions are among several factors contributing to fungal growth and aflatoxins contamination in maize, leading to significant economic loss, reduced household income, health problems to humans and animals and interferes with food security to communities. Structured questionnaires were used to collect information on awareness associated with aflatoxin contamination in maize from 160 smallholder farmers, 160 consumers and 60 traders in Kondoa and Chemba districts in Dodoma Region. A total of 90 maize samples (40 from smallholder farmers, 30 from consumers and 20 from traders) were analyzed for AFB1 using immuno-affinity high-performance liquid chromatography (HPLC) type Agilent Technologies 1200 serial. Data were statistically analyzed to assess awareness levels among maize main stakeholder and to check the current levels of aflatoxins B_1 contamination in the study community. AFB_1 was detected in five samples. About 3.3% of the contaminated maize had AFB_1 levels above TBS acceptable levels (5 µg/kg). The highest mean concentration of AFB1 was in maize samples taken from traders with a mean of 9.88±5.904 µg/kg. The majority 56% of smallholder farmers and 52% of traders were aware of aflatoxins contamination and associated health effects on animals and humans. However, 74% of consumers were unaware of aflatoxins contamination in maize. The levels of contamination are low in the sample taken along maize value chain. An effective and broad awareness programme for community especially consumers on good management for prevention of aflatoxins contamination is necessary, as maize is the most consumed grain in the study area.

Key words: Aflatoxins contamination, smallholder farmers, consumers.

INTRODUCTION

Agriculture accounts for 26.7% of Tanzania's GDP and provides employment for majority of the nation's population (FAO, 2020). The safety of food is a pervasive concern of general public health and government authorities' worldwide (Logrieco et al., 2018). However, fungi producing a poison that contaminates foods crops are often found on the most important staple crops. Increasing awareness of its occurrence and contamination

is important to all stakeholders due to adverse effects on human and animal health (Wild et al., 2012). Fungi are capable of producing hundreds of secondary metabolites but only a relative few are regulated (Ostry et al., 2017). These metabolites include the widely regulated mycotoxins such as aflatoxin, fumonisins, trichothecenes (particularly deoxynivalenol), ochratoxins and zearalenone. Other mycotoxins that are less regulated include the ergot alkaloids, patulin and the T-2 and HT-2 toxins (Logrieco et al., 2018). The three main genera of fungi that produce mycotoxins and toxigenic are *Aspergillus*, *Fusarium*, and *Penicillium*, that attack various food commodities. *Aspergillus* spp. is fungi that produce a group of toxins known as aflatoxin (Guchi, 2015). Specifically, *A. flavus* is the major aflatoxin producing species, which predominately contaminates maize (Samson et al., 2014; Iqbal et al., 2015; Seetha et al., 2017). Aflatoxins B_1 (AFB_1), the most potent of the aflatoxin is classified as a human carcinogen (Adekoya et al., 2017) and has been associated with child growth impairment, suppressed immune function, and death due to acute poisoning known as aflatoxicosis (Salano et al., 2016; Shirima et al., 2015). In 2016, death resulting from acute aflatoxicosis has also been reported in Tanzania and there were 68 cases of acute aflatoxicosis and 20 related deaths in central Tanzania (Manyara and Dodoma) (Kamala et al., 2018). In Tanzania, maize is the most important staple crop for the majority of the population and a major component of feed for livestock (URT, 2016). Smallholder farmers produce over 85% of the total national cultivation of maize, and production is growing at an average annual rate of 6.44% in 2020 (URT, 2020); it also serve as a source of 30% of dietary calories to millions of population (FAOSTAT, 2020). The majority of smallholder farmers produce maize as food and cash crop while consumers prefer white dent corn with a negligible amount of yellow corn grown in Tanzania (Mtaki, 2019). Thus, maize is important and therefore deserves adequate and effective monitoring in its production chain (Nyirenda et al., 2021).

A recent review suggests that about 60 to 80% of the global food crops are contaminated with mycotoxins (Eskola et al., 2020). This estimation pushed back the widely cited 25% estimation attributed to the Food and Agricultural Organization (FAO) of the United Nations. Nonetheless, these figures are surprising because a large proportion of the world's population is faced with the risks associated with exposure to aflatoxins causing significant economic losses (Wu, 2015); interfered with food security; significant decline in agricultural trade between developed and developing countries (WHO, 2018). In many developing countries, levels of aflatoxins awareness are extremely low or non-existent altogether. Awareness has been found to vary with various socioeconomic characteristics. For instance, in Tanzania, studies have shown that education level has a positive effect on aflatoxins awareness (Ngoma et al., 2017; Magembe et al., 2017). In Kenya, women were found more informed of the danger of fungal toxins and cautious to moldy feeds than men (Kiama et al., 2016). Furthermore, in Vietnam, young farmers (at age of 21–29) were more informed of aflatoxins in crops than the older groups (Lee et al., 2017). The field of study particularly life sciences had a positive impact on aflatoxins awareness in Ghana (Ayo et al., 2018) while individuals in other occupations are more informed of aflatoxins than farmers in Ethiopia (Ephrem et al., 2014). Detection and quantification of aflatoxins levels in human food are important to compare levels of contamination with the recommended maximum residue limit (MRL), so that appropriate remedial action and preventive practices of aflatoxins contamination during handling and storage of foods can be implemented (Udomkun et al., 2017). Aflatoxins contamination in maize can only be accurately quantified with laboratory testing along maize value chains, and hence significantly reduce risks of aflatoxins exposure (Hoffmann et al., 2018). Therefore, the study aimed at assessing awareness of aflatoxins among stakeholders and determining the current levels of aflatoxin in maize stored among stakeholders in Chemba and Kondoa districts of Dodoma region.

MATERIALS AND METHODS

Study design, sampling procedure and sample collection

A cross-sectional descriptive study was carried out between smallholder maize farmers (have less than 5 acres), traders (Village Agents, wholesaler) and consumers (different professions, (farmers, teachers, students, house wife and entrepreneurs) in collecting field data in Kondoa and Chemba districts, whereby two wards in each district were selected. Then two villages were selected in each ward to make a total of eight villages. A simple random sampling was used to select 40 samples from smallholder farmers, 30 samples from consumers and 20 samples from traders making a total of 90 samples. Face to face interview was among selected 20 smallholder farmers, 20 consumers from each village, making a total of 160 smallholder farmers and 160 consumers' respondents. On the other hand, 60 traders including market sellers were randomly selected from the study area. A total of 90 maize samples were purchased and collected randomly from three different stakeholders (smallholder farmers, 40 samples; consumers, 30 samples; and traders, 20 samples) in the study area. The larger number of maize sample collected is due to availability of the samples from stakeholders. All samples were coded and transported in an ice box together with their original packaging prior to laboratory analysis at Tanzania Bureau of standards (TBS) in Dar es Salaam.

*Corresponding author. E-mail: a_ndwatta@yahoo.co.uk. Tel: +255 712209528.

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

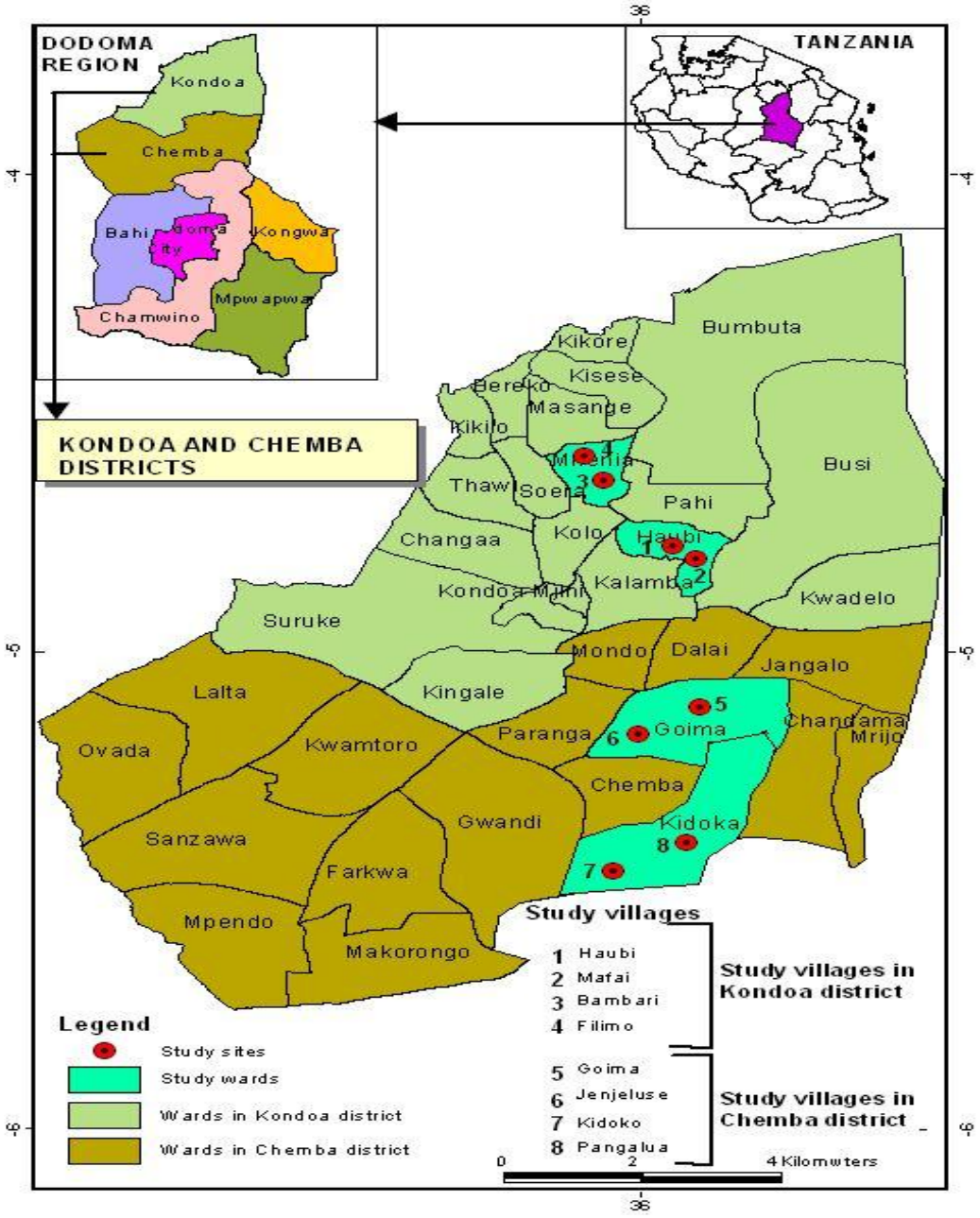

Figure 1. Map showing study sites in Kondoa and Chemba districts of Dodoma region.
Source: Authors

Study area

The study was conducted during the 2020-2021 cropping season in the semi-arid agro-ecological zone (Kondoa and Chemba districts) of Dodoma Region (Figure 1). Kondoa District lies between latitude 4° 12` to 5° 38` south and longitude 35° 6` to 36° 2` East. Chemba District lies between 5° 14` to 36° 00` south and longitude 35° 53 to 24° 00 East. Its climate is wet savannah characterized by a long dry season (DEPRP, 2012). The districts were selected due to physical attribute and multiple threats experienced annually rendering their communities at risk. The main threats affecting the districts include drought, deforestation, soil degradation and hunger conditions which impose a pattern of risk evasion in traditional agriculture (URT, 2017). Furthermore, the reported epidemic of aflatoxicosis in 2016 (Kamala et al., 2018) and the presence of the conditions conducive to the formation of aflatoxins production is another issue (Ngoma, 2019).

Sample size estimation

Since the exact population of maize main stakeholders (smallholder farmers, traders and consumers) was unknown, the sample size was estimated using the Kothari equation (Kothari and Garg, 2014):

$n = z^2P (1-P) / e^2$

Where; n = sample size, Z = Standard variant at a given confidence level, for this study a 95% confidence level = 1.96, P = Standard deviation that will show how much the results will vary from each other and the mean number for this study (0.5) was used and e = acceptable error (the precision/ estimation error) set at 5% (0.05) for this study. Thus, the sample size of the study for assessment of awareness among stakeholders was:

$n = 1.96^2 \times 0.5 (1 - 0.5)/0.05^2$

n = 384 for respondents for interview

And for samples used in determining the aflatoxins contaminations, maximum allowable error of 0.05% was used thus, the sample size of maize for analysis was:

$n = 1.96^2 \times 0.05 (1 - 0.05)/0.045^2$

n = 90 for maize sample for aflatoxin analysis

Data collection tools

The household survey was conducted using a pretested structured questionnaire. Face-to-face interviews were conducted with randomly selected stakeholders (smallholder farmers, traders and consumers). The data of the study was collected using quantitative methods.

Aflatoxins analysis

Chemicals and standards, HPLC conditions and column and other materials

HPLC grade chemicals, acetonitrile, methanol and glacial acetic acid were from Fisher Chemical, UK. Aflatoxins standards (2.02 µg/kg for AFB_1 and AFG_1, 0.505 µg/kg for AFB_2, and AFG_2) solution were of chromatography grade obtained from Biopure, Romer Labs Diagnostics GmbH-Tulin Austria, Distilled water was produced with a Milli-Q Integral 15 water purification system - France and Immunoaffinity columns (AflaTest from Romer Labs GmbH, Technopark 5and 3430 Tulin, Austria).

HPLC conditions

HPLC with a fluorescence detector (FLD) (Model Agilent ChemStation technology, series 1200, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA). The HPLC system was equipped with a G1322A degasser, and a G1311A Quat pump. Chromatography separation was achieved by Zorbax 20 Rbax RX C18 column 5 µL (250 × 4.6 mm) (Agilent, USA) and maintained at 30°C and a flow rate of 1.2 ml/min. The analytical separation of aflatoxins (AFB1, AFB2, AFG1 and AFG2) was performed using the mobile phase contained water: methanol: acetonitrile (60:30:10, v/v) for both standard solution and sample extracts. After separation, AFG1 and AFB1 were derivatized to allow their detection with a fluorescence detector at an emission wavelength of 465 nm and an excitation wavelength of 360 nm.

Extraction of samples

Maize grain was ground separately to obtain a homogenous flour mixture and then sub-divided to obtain representative sub-samples for analysis. Each ground maize sample (Maize flour) or quality control samples were placed into amber colored Erlenmeyer flask and weighed using the calibrated analytical balance to 25 ± 0.1g (Shimadzu electronic balance, ATX224 type). By using a measuring cylinder, 100 ml of methanol: water (70:30 v/v) as extraction solvent was added to the 250 ml amber colored Erlenmeyer flask containing the sample. The flask was placed on the gyratory shaker (Stuart® Orbital Shaker SSL1, Cole-Parmer LLC, and USA) at 250rpm/30 min, then using a filter paper Whatman No. 1, the extract was filtered into a 250 ml flask.

Dilution stage

Four (4) ml of extract sample was transferred to 15 ml amber colored volumetric flask, followed by the addition of 8 ml of distilled water. Then, the mixture was vortexed (Talboys® Hvy Dty Vortex, USA) for 1 minute to get a homogeneous mixture.

Clean-up of aflatoxins

The diluted extract was loaded and allowed to pass through Solid Phase Extraction (SPE) immunoaffinity columns and the sample loaded columns were rinsed twice with 10 ml of HPLC grade water.

Elution stage

The adsorbed aflatoxins were eluted with 1 ml of HPLC grade methanol and the eluent was collected in HPLC vials. Finally, the pressure was slightly applied on top of the column to remove any remaining liquid. Three hundred microliter of the eluate was mixed with 0.6 ml of water and 0.1 ml of acetonitrile and the mixture was vortexed for 30 seconds ready for HPLC injection.

Determination of the limit of detection (LOD) and limit of quantification (LOQ) of the HPLC method

The LOD and LOQ were established by analyzing successive lowest dilutions (0.1 µg/kg) of the standard solution in the matrix. These LOD and LOQ values were related to the signal to noise ratio considering the concentration generated at 3 and 10 times, respectively of the lowest calibration point. The limits of detection (LOD) and quantification (LOQ) of the HPLC method for AFB1, AFB2, AFG1 and AFG2 were 0.1 and 0.5 µg/kg, respectively. The precision of the method was determined by running the lowest standard of 0.1 ng/mL ten times for three days and precision was determined by calculating their relative standard deviation. The measurement uncertainty, expressed as relative standard deviation (RSD) was 1.402% and this is within the acceptable range of < 2.4%, ISO 16050:2003.

Data analysis

Statistical Package for Social Sciences (IBM SPSS® Version 20, Minnesota and USA) was used to analyze the obtained data. The analysis involved descriptive statistics to describe the sample population, socio-demographic of respondents and awareness of aflatoxins contamination of maize. The chi-square test was used for testing the association between study independent variables and dependent variable (aflatoxins contamination). Laboratory analysis data was entered and processed using Excel sheets and analyzed using R software (version 4.1.0, 2021) whereby Friedman's test was used to test for significant differences between the combination

Table 1. Socio-demographic characteristics of interviewed respondents (n=380).

Variable	Descriptions	(%) respondents		
		Farmers (n= 160)	Consumer (n = 160)	Traders (n = 60)
Districts	Kondoa	50	50	58
	Chemba	50	50	42
Gender	Male	55	59	89
	Female	45	41	13
Age categories	20 - 35	20	48	32
	36 - 45	26	19	46
	46 - 55	28	24	18
	55 < above	25	9	3
Education level	Informal education	6	9	0
	Primary education	88	67	70
	Secondary education	5	19	30
	Tertiary education	0	4	0
	University level	0	6	0
Marital status	Married	97	88	88
	Single	3	12	12

Source: (Author survey, 2021).

of the type of stakeholder and districts in aflatoxins concentration from the maize grain samples. A probability value less than 0.05 was considered significant and the mean separation test was done using the Turkey HSD test.

RESULTS

Recovery of aflatoxins B_1 contamination

The recovery of aflatoxin B_1 were greater than 70% (94.025, 93.09 and 92.2%) with an average of 93.11%, indicating the suitability and good performance of the HPLC, extraction protocol and quantification (Beyene et al*.,* 2019)

Social - demographic characteristics of respondents

Results in Table 1 show the socioeconomic characteristics of the respondents. Over 90% were married giving an indication of the importance of the marriage in the study area. About 75% of all stakeholders that is smallholder farmers, traders and consumers completed at least primary school education indicating a measure of literacy.

Stakeholders' level of awareness on aflatoxins in maize contaminations

The overall score (Figure 2) indicate that more smallholder farmers and traders and a few consumers are aware of the occurrence, cause and effect of aflatoxins contamination in maize in Kondoa and Chemba districts.

Aflatoxins contamination in maize samples

The mean values of aflatoxins AFB_1 and total aflatoxins in farmer, traders and consumer maize samples ranged from 0.00±0.000 to 9.88±5.904 as shown in Table 2. The highest mean value for total aflatoxins was in traders' maize samples. However, there was a significant difference between the means at $p<0.05$.

A higher number of samples were taken from smallholders farmers due to the availability of samples that is normally stored for sale at a higher price later. Mean ± SEM across the column with different statistical letters indicates statistical difference according to the Turkey HSD test.

Incidence of aflatoxins B_1 contamination in maize grain samples that exceeding EU and TBS regulatory limits

Few samples were contaminated with AFB_1 (Figure 3), Samples from Filimo and Mafai wards did not detect to AFB_1 and total aflatoxins. Also Jengeluse and Goima wards didn't detect for aflatoxins B_1 contaminations.

Table 2. Mean aflatoxins concentration (µg/kg) in maize grains samples collected from different stakeholders in Kondoa and Chemba Districts (Mean ± SEM).

Stakeholder	District	Sample (N)	Aflatoxins B1 Mean ±SEM (µg/kg)	Total aflatoxins Mean SEM (µg/kg)
Consumer	Chemba	15	0.00±0.000[b]	0.00±0.000[b]
	Kondoa	15	0.00±0.000[b]	0.00±0.000[b]
Smallholder Farmer	Chemba	20	0.04±0.029[b]	0.04±0.029[b]
	Kondoa	20	0.00±0.000[b]	0.00±0.000[b]
Trader	Chemba	10	0.00±0.000[b]	0.00±0.000[b]
	Kondoa	10	9.88±5.904[a]	12.42±7.652[a]

Source: Authors

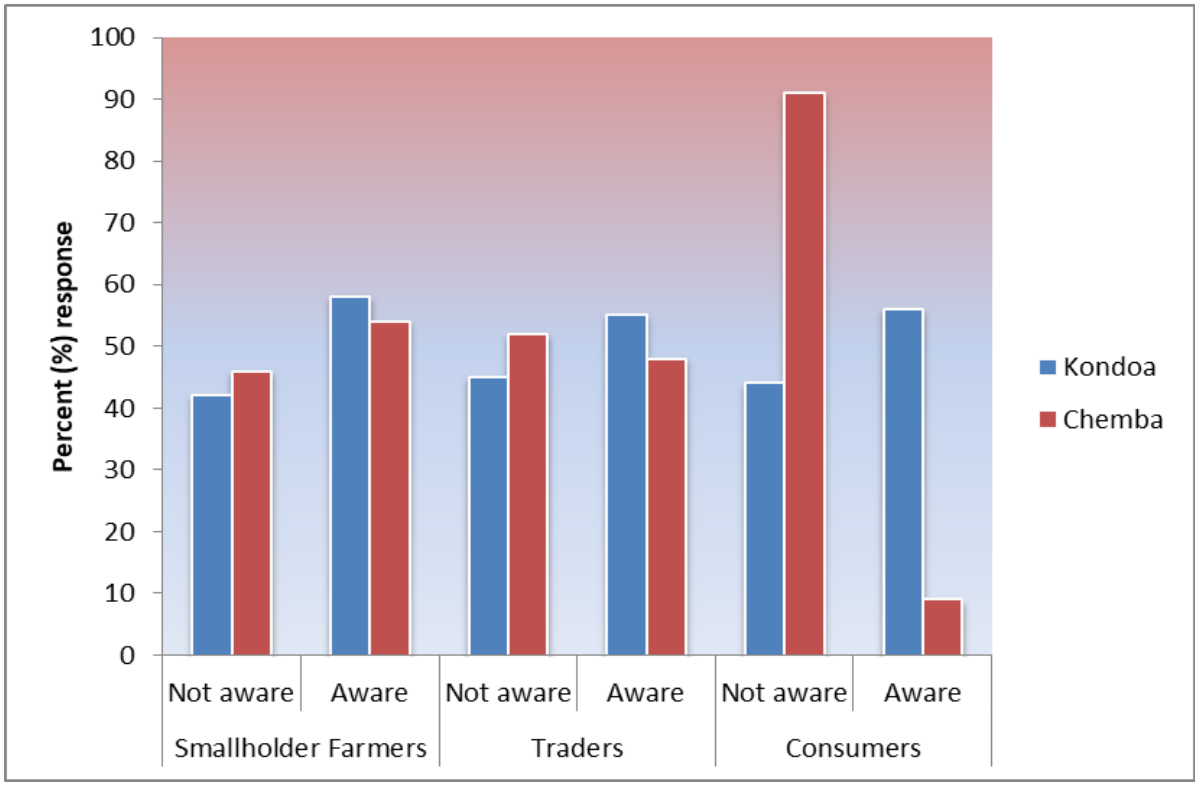

Figure 2. Respondents' overall score on awareness of aflatoxins contamination in maize.
Source: Authors

DISCUSSION

Social - demographic characteristics of respondents

Generally, the study found that the number of males who participated in the study exceeded that of female. The male participants were 61% (Smallholder farmers 55%, Traders 89% and Consumer 59%) (Table.1) while the female participants were 39%, this implied that male respondents were dominating the main supply chain. In the study area traditional farming activities are dominated by women because it's a tedious work. Women in nature are tolerant as being seen in the way of taking care of the family hence, traditional believed that farming activities are women work. Lack of permanent market to sell maize was the reasons for men to engage in trading activities. Male respondents were dominating in trading activities, a trend found mostly in many developing countries actively engaged in trading activities and in providing information. A similar trend was observed by Toma (2019) in Ethiopia

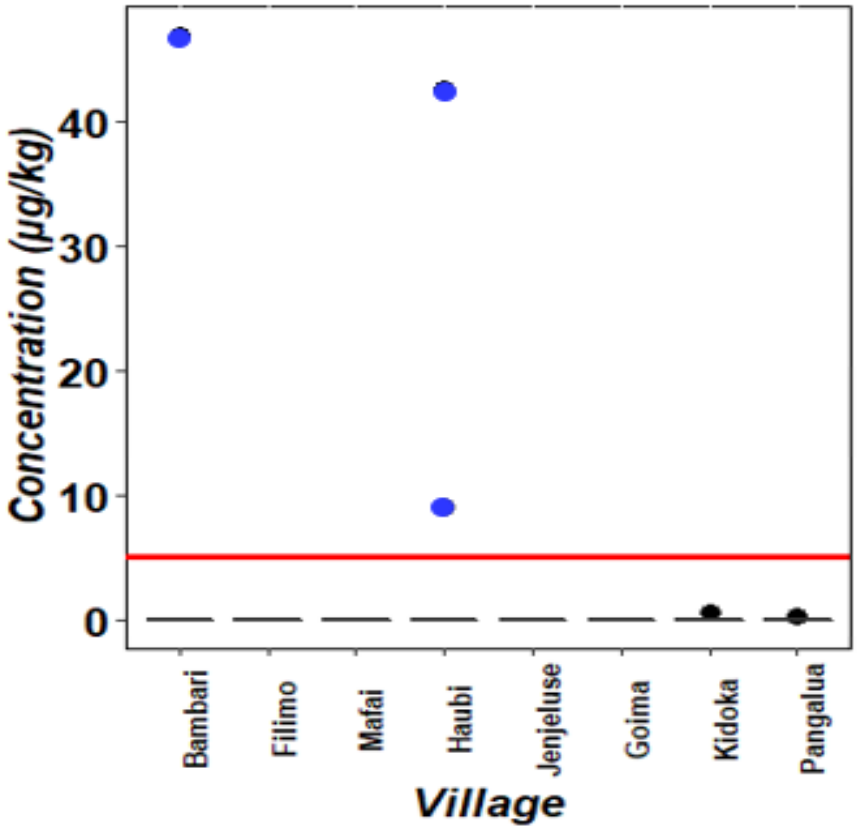

Figure 3. Incidence of aflatoxins B_1 contamination in maize grain samples that exceeding TBS regulatory limits.
Source: Authors

who found that farming activities and trades are dominated by males; the study also noted that more than half (53%) of smallholder farmers were aged above 45 years of age. On the other hand, the majority (78%) of traders in the study area were aged between 36 – 45 while, the mean duration of involvement in the maize business was 8 years; Most (67%) of consumers were in the age group between 20 to 45 years old. This finding implies that maize value chain is a demanding activity; therefore those involved ought to be physically energetic and able to supply the required labour so as to meet their responsibilities and goals. Descriptive statistics showed that the majority (88%) of smallholder farmers interviewed had primary school education, 70% of traders had attained primary school education; while 67% of consumers had attained primary school education. These findings show that farmers, traders and consumers had at least a basic primary level of education. These imply that the majority of respondents were able to follow training and instructions as they could read and write in Kiswahili. Education may help them read and understand guidelines associated with occurrence, causes, health effects and prevention of aflatoxins contaminations. These findings conform to the study by Aulakh and Regmi (2013) who suggested that smallholder farmers and traders with at least basic education are needed to reduce food losses.

Stakeholders' level of awareness on aflatoxins in maize contaminations

This study revealed that level of education was directly related to aflatoxins contamination awareness. Maize value chain is highly dominated by Smallholder farmers, whose education level was primary school (88%) and very few respondents (<10%) in this category did not hear of aflatoxins contaminations in their lifetime. Awareness of aflatoxins contamination in maize was high among smallholder farmers (58%) and traders (55%), while it was low (42%) among consumers in Kondoa District. Similarly, smallholder farmers' awareness was 54%, traders 48% and the lowest (9%) among consumers in Chemba District. The stakeholder farmers' knowledge of aflatoxin in a large amount is attributed to farmer field schools and training conducted with agricultural extension officers in the study area. Similar studies by Kamala et al. (2016) and Hell and Mutegi (2011)

reported training to improve maize smallholders' farmers' awareness of fungi and aflatoxin contamination. According to Massomo (2020), the high level of awareness found in the area is attributed to the information that was communicated on contamination of food commodities, acute poisoning and deaths due to aflatoxins, during the outbreak in 2016. However, this conclusion is contrary to the studies done in Tanzania by Degraeve et al. (2016), Magembe et al. (2016) and Shabani et al. (2015) who found low level of awareness before the outbreak of the death related to aflatoxins. Traders scored higher than consumers may be due to regular training on aflatoxins contamination, seminar and workshops. Similar observations were reported by James and Zikankuba (2018) that training, seminar and workshops on aflatoxins increase awareness of maize traders. Likewise, a study conducted in Kenya found that most (56.6 %) traders were aware of aflatoxin contamination (Nyangaga, 2014). Furthermore, analysis shows that consumers (this categories mixed up with different field of people such as smallholder farmers (72%), primary school teachers (10%), secondary school student (10%) and entrepreneur, housewife were (<8%) had low awareness compared to other groups. Possible explanation for this observation is clearly depicted in this study. Education was an important mode of dispensing information and knowledge on aflatoxins contamination to public. This observation reflects Kamala et al. (2018) and Ezekiel et al. (2013) who reported the lowest (15%) level of consumers' awareness of aflatoxins contamination. This implies low public awareness of aflatoxins contamination affects mainly people from remote areas who have less access to information on aflatoxins as compared to those in urban areas. Respondents from Kondoa District were more aware compared to Chemba respondents, this is not unique as previous studies (Kimanya et al., 2014; Magembe et al*.,* 2016) reported that in Tanzania, awareness of aflatoxins and health impacts varied between districts. The finding implies that the presence of projects dealing with aflatoxins in the districts and stakeholders' commitment and ability to implement the practice might have contributed to this awareness.

Aflatoxins contamination in maize samples

Findings in this study reveal the significant occurrence of important aflatoxins in main actors' samples in these districts maize supply chain. This is important because maize is dietary staple food in these districts affected by the aflatoxicosis outbreak, aflatoxins contamination from traders' samples therefore, is an important public health concern and these toxins may pose significant human health risks that may be increased by occurrence in the diet. Table 3 indicates that out of 90 maize grain samples collected from various villages in three different stakeholders in the maize value chain from the study area, five (5) samples were contaminated with aflatoxins B_1. Moreover, a high prevalence with AFB_1 and total aflatoxins were found in the samples taken from traders, there were low concentration detected in samples from smallholders' farmers while none of the consumers' samples was detected for aflatoxins contamination. The lower levels of aflatoxins contamination in farmers' maize samples probably was due to environmental conditions, such as change in temperature and relative humidity of surrounding as well as a good type of soil, since the moulds live in soil, surviving off dead plant and animal matter, but do spread through the air via airborne *conidia are* the natural factors that influence aflatoxins incidence during maize production (Atanda et al*.,* 2013) good farmers' practices such as timely harvesting, ensuring uniform drying of maize to a safe moisture level and proper storage is critical in the maize value chain. Storage at less than 13% moisture content, 65% relative humidity and temperature of less than 25^0C prevents the growth of storage moulds (Ademola et al*.,* 2021). Despite contamination increases with time in storage, the majority of the samples used in the analysis were stored in good condition for eight months at the farmers' store (Monyo et al., 2012; Ezekiel et al*.,* 2013). The samples collected from traders demonstrate that mean levels of aflatoxins B_1 in stored maize was significantly higher compared to other actors (smallholder farmers and consumers). The drastic increase in aflatoxins probably was because traders usually purchase maize from different locations, different storage facilities as well as different maize varieties, which may also have aflatoxins contamination. Frequent opening and improper closing of the storage facilities could also add moisture from the atmosphere and thus the quality of dried grain be affected by the variation in final moisture content during storage. Besides, efforts to address the issue of aflatoxins prevention programs is geared very much to smallholder farmers and not traders and consumers. The prevalence of aflatoxins contamination obtained in trader's samples was significantly high which indicates the risk of chronic exposure to the consumers. The findings are similar to the study by Oyekale and Oladele (2012) who noted that traders' maize samples were contaminated with higher mean levels of aflatoxins B_1. Therefore, to ensure high quality during storage, maize should be protected from weather, growth of microorganisms, and insects (Oyekale and Oladele*,* 2012).

AFB_1 has been detected more frequently compared to other types of aflatoxins, similar to what was reported by Kachapulula et al. (2017) in Zambia that maize samples were contaminated with aflatoxins by 5%. The results of the present study were significantly lower than the study conducted by Dos Santos et al. (2013) in Brazil where 16% of the maize samples from farmers were contaminated with aflatoxins B_1 and contrary to Kaale et al. (2021) who report high aflatoxins B_1 contaminations in

Table 3. Percentage of maize contaminated with aflatoxins in Kondoa and Chemba.

Stakeholder	District	Sample(N)	Sample contaminated with aflatoxins B1	
			n	%
Consumer	Chemba	15	0	0.0
	Kondoa	15	0	0.0
Smallholder Farmer	Chemba	20	2	10.0
	Kondoa	20	0	0.0
Trader	Chemba	10	0	0.0
	Kondoa	10	3	30.0
Total		**90**	**5**	**5.6**

N is the total number of samples analyzed from two different districts and from different stakeholders (smallholder farmers, Traders and Consumers) and n is total number of contaminated samples from each district and from each stakeholder.
Source: Authors

maize samples. Three samples, which were all taken from Bambari and Haubi village in Kondoa District were found to be contaminated with aflatoxins B_1, exceeded the acceptable limits for aflatoxins B_1 of 5 µg/kg (TBS, 2018) with maximum concentrations of 46.99 µg/kg (Figure 3) and the concentrations were 42.69,10.11 and 46.99 µg/kg. Furthermore, high levels can occur if rodents and other pest attack and damage maize grain and if storage occurs under unfavorable conditions over long periods of storage. Two samples (2) of contaminated maize (Figure 3) from Kidoka and Pangalua villages in Chemba Districts were found to be below (5 µg/kg) acceptable TBS regulatory limits for AFB_1 and concentrations were 0.29 and 0.51 µg/kg. This supports a study by Ezekiel and Sombie (2014) in Nigeria which found that aflatoxins were present at the internationally recommended level for aflatoxins B_1 and total aflatoxins in the maize sample. Thus, the results indicated that consumers of maize in this area have been at significant risk for exposure to low levels of aflatoxins contaminations. The present study found low aflatoxins contamination at samples from farmers at levels below the maximum tolerated limit (MTL). Similar to the studies reported by Bonni et al. (2021) in Tanzania, and Kamika and Tekere (2016) in Congo whose findings indicated a low mean concentration of AFB_1 in maize samples. These observations might be a result of proper is result storage of maize along the maize value chain. Storage at less than 13% moisture content, 65% relative humidity; and temperature of less than 25°C prevents the growth of molds.

CONCLUSIONS AND RECOMMENDATION

The study shows that few samples were contaminated with AFB_1; however high AFB_1 levels were found in trader's sample which was above the recommended Tanzania Bureau of Standards (TBS) regulatory limit. A significant number of smallholder farmers and traders stakeholders in Kondoa and Chemba district in Dodoma Region were aware of aflatoxins contamination in maize, which is vital in improving food safety in the country. However, consumers in the research area have extremely low awareness level of aflatoxins contamination, which increases the risks of aflatoxins contamination along the maize value chains. Therefore, there is a need of introducing method of identifying and managing food safety risk and food safety program, Hazard Analysis Critical Control Point (HACCP), among stakeholders which can provide assurance to customer, the public and regulatory agencies of food safety in the country. The study recommends an urgent development of an effective and broad community awareness programme on aflatoxin contaminations in maize on occurrence, causes and health effects in humans. It is important that consumers and all stakeholders along maize value chain be educated on the potential harmful effects on AFB_1 on human health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adekoya I, Njobeh P, Obadina A, Chilaka C, Okoth S, De Boevre M, De Saeger S (2017). Awareness and prevalence of mycotoxin contamination in selected Nigerian fermented foods. Toxins 9(363):1-16.

Ademola O, Turna NS, Liverpool-Tasie LSO, Obadina A, Wu F (2021). Mycotoxin reduction through lactic acid fermentation: Evidence from commercial ogi processors in southwest Nigeria. Food Control 121:107620-107627.

Aulakh J, Regmi A (2013). Post-harvest food losses estimation-development of consistent methodology. https://www.fao.org/ess/GS_SAC_2013. Google Scholar

Atanda O, Makun HA, Ogara IM, Edema M, Idahor KO, Eshiett ME, Oluwabamiwo BF (2013). Fungal and mycotoxin contamination of Nigerian foods and feeds. Mycotoxin and Food Safety in Developing Countries 68:1455-1458.

Ayo EM, Matemu A, Laswai GH, Kimanya ME (2018). Socioeconomic characteristics influencing level of awareness of aflatoxins contamination of feeds among livestock farmers in Meru district of Tanzania. Scientifica, pp. 1-11.

Beyene AM, Du X, E Schrunk D, Ensley S, Rumbeiha WK (2019). High-performance liquid chromatography and enzyme-linked immunosorbent assay techniques for detection and quantification of aflatoxin B 1 in feed samples: a comparative study. BMC Research Notes 12(1):1-6.

Bonni SB, Beed F, Kimanya ME, Koyano E, Mponda O, Mamiro D, Mahuku G (2021). Aflatoxin contamination in Tanzania: quantifying the problem in maize and groundnuts from rural households. World Mycotoxin Journal 14(4):1-12.

Degraeve S, Madege RR, Audenaert K, Kamala A, Ortiz J, Kimanya M, Haesaert G (2016). Impact of local pre-harvest management practices in maize on the occurrence of Fusarium species and associated mycotoxins in two agroecosystems in Tanzania. Food Control 59:225-233.

DEPRP (2012). Kondoa District Emergency preparedness and response plan. https://Ijisrt.com/wp-content/uploads/2019/04/IJISRT19MA167.pdf

Dos Santos JS, Souza TM, Ono EYS, Hashimoto EH, Bassoi MC, de Miranda MZ, Hirooka EY (2013). Natural occurrence of deoxynivalenol in wheat from Paraná State, Brazil and estimated daily intake by wheat products. Food Chemistry 138(1):90-95.

Ezekiel CN, Sulyok M, Frisvad JC, Somorin YM, Warth B, Houbraken J, Odebode AC (2013). Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. International Journal of Food Microbiology 162(3):231-236.

Ezekiel CN, Sombie JI (2014). Survey of aflatoxins and fungi in some commercial breakfast cereals and pastas retailed in Ogun State, Nigeria. National Science 12(6):27-32.

Ephrem GA, Amare D, Mashilla K, Mengistu A, Chemeda F (2014). "Stakeholders' awareness and knowledge about aflatoxin contamination of groundnut (arachis hypogaea L.) and associated factors in eastern Ethiopia. Asian Pacific Journal Tropical Biomedical 4(1):930-937.

Eskola M, Kos G, Elliott CT, Hajšlová J, Mayar S, Krska R (2020). Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate of 25%. Critical Reviews in Food Science and Nutrition 60 (16):2773-2789.

Food and Agriculture Organization (FAO) (2020). Tanzania at a glance. Food and Agriculture Organization of the United Nations. http://www.fao.org/tanzania/fao-in-tanzania/tanzania-at-aglance/en/ visited on 10/05/2022

FAOSTAT (2020). Crop yield in Tanzania 2020. https://www.fao.org/faostat/en/#data/QP. Google scholar

Guchi E (2015). Aflatoxin contamination in groundnut (Arachis hypogaea L.) caused by Aspergillus species in Ethiopia. Journal of Applied and Environmental Microbiology 3(1):11-19.

Hell K, Mutegi C (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. African Journal of Microbiology Research 5(5):459-466.

Hoffmann V, Jones K, Leroy JL (2018). The impact of reducing dietary aflatoxin exposure on child linear growth: a cluster randomised controlled trial in Kenya. BMJ Global Health 3(6):1-10.

Iqbal SZ, Jinap S, Pirouz AA, Faizal AA (2015). Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review. Trends in Food Science and Technology 46(1):110-119.

James A, Zikankuba VL (2018). Mycotoxins contamination in maize alarms food safety in sub-Sahara Africa. Food Control 90:372-381.

Kaale LD, Kimanya ME, Macha IJ, Mlalila N (2021). Aflatoxins contamination and recommendations to improve its control: a review. World Mycotoxin Journal 14(1):27-40.

Kachapulula PW, Akello J, Bandyopadhyay R, Cotty PJ (2017). Aflatoxin contamination of groundnut and maize in Zambia: observed and potential concentrations. Journal of Applied Microbiology 122(6):1471-1482.

Kamala A, Kimanya M, Haesaert G, Tiisekwa B, Madege R, Degraeve S, De Meulenaer B (2016). Local post-harvest practices associated with aflatoxin and fumonisin contamination of maize in three agro-ecological zones of Tanzania. Food Additives and Contaminants 33(3):55-559.

Kamala A, Shirima C, Jani B, Bakari M, Sillo H, Rusibamayila N, Simba A (2018). Outbreak of an acute aflatoxicosis in Tanzania during 2016. World Mycotoxin Journal 11(3):311-320.

Kiama TN, Lindahl JF, Sirma AJ, Senerwa DM, Waitangi EM, Ochungo PA. Grace D (2016). Kenya dairy farmer perception of moulds and mycotoxins and implications for exposure to aflatoxins: A gendered analysis. African Journal of Food, Agriculture, Nutrition and Development 16(3):11106-11125.

Kimanya ME, Shirima CP, Magoha H, Shewiyo DH, De Meulenaer B, Kolsteren P, Gong YY (2014). Co-exposures of aflatoxins with deoxynivalenol and fumonisins from maize-based complementary foods in Rombo, Northern Tanzania. Food Control 41:76- 81.

Kamika I, Tekere M (2016). Occurrence of aflatoxin contamination in maize throughout the supply chain in the Democratic Republic of Congo. Food Control 69:292-296.

Kothari CR, Garg G (2014). Research Methodology. Methods and Techniques. Third Edition. New Age International Publishers, India. p 102.

Lee HS, Nguyen-Viet H, Lindahl J, Thanh HM, Khanh TN, Hien LTT, Grace D (2017). A survey of aflatoxin B1 in maize and awareness of aflatoxins in Vietnam. World Mycotoxin Journal 10(2):195-202.

Logrieco AF, Miller JD, Eskola M, Krska R, Ayalew A, Bandyopadhyay R, Leslie JF (2018). The Mycotox Charter: Increasing awareness of, and concerted action for, minimizing mycotoxin exposure worldwide. Toxins 10(4):149.

Magembe KS, Mwatawala MW, Mamiro DP, Chingonikaya EE (2016). Assessment of awareness of mycotoxins infections in stored maize (Zea mays L.) and groundnut (*Arachishypogea L*.) in Kilosa District, Tanzania. International Journal of Food Contamination 3(1):1- 8.

Magembe KS, Mwatawala MW, Mamiro DP, Chingonikaya EE (2017). Assessment of awareness of mycotoxins infections in stored maize (Zea mays L.) and groundnut (Arachishypogaea L.) in Kilosa District, Tanzania. International Journal of Food Contamination 4(1):1-8.

Massomo SM (2020). Aspergillus flavus and aflatoxin contamination in the maize value chain and what needs to be done in Tanzania. Scientific African 10:1-17.

Monyo ES, Njoroge SMC, Coe R, Osiru M, Madinda F, Waliyar F Anitha S (2012). Occurrence and distribution of aflatoxin contamination in groundnuts (Arachis hypogaea L) and population density of Aflatoxigenic Aspergilli in Malawi. Crop Protection 42:149-155.

Mtaki B (2019). *Tanzania Corn, Wheat and Rice Report.* Global Agricultural Information Network, USA. 11pp.

Ngoma SJ, Kimanya M, Tiisekwa B (2017). Perception and attitude of parents towards aflatoxins contamination in complementary foods and its management in central Tanzania. The Journal of Middle East and North Africa Sciences 3(3):6- 21.

Ngoma SJ (2019). The influence of awareness, knowledge and practices of communities on childhood dietary exposure to aflatoxins in Central Regions of Tanzania. Thesis for Award of PhD Degree at Sokoine University of Agriculture, Morogoro, Tanzania. P. 330.

Nyangaga DN (2014). Traders' awareness and level of aflatoxins in human foods and cattle feed in selected markets and stores in Nairobi County, Kenya. Dissertation for Award of MSc Degree at Kenyatta University, Nairobi, Kenya, p 133.

Nyirenda H, Mwangomba W, Nyirenda EM (2021). Delving into possible missing links for attainment of food security in Central Malawi: Farmers' perceptions and long term dynamics in maize (Zea mays L.) production. Heliyon 7(5):e07130.

Oyekale AS, Oladele OI (2012). Determinants of climate change adaptation among cocoa farmers in southwest Nigeria. Journal of Science and Technology 2(1):154-168

Ostry V, Malir F, Toman J, Grosse Y (2017). Mycotoxins as human carcinogens the IARC Monographs classification. Mycotoxin Research 33(1):65-73.

Salano EN, Obonyo MA, Toroitich FJ, Omondi B, Aman BO (2016). Diversity of putatively toxigenic Aspergillus species in maize and soil samples in an aflatoxicosis hotspot in Eastern Kenya. African Journal of Microbiology Research 10(6):172-184.

Samson RA, Visagie CM, Houbraken J, Hong S-B, Hubka V, Klaassen CHW, Perrone G, Seifert KA, SuscaA , Tanney JB, Varga J, Kocsubé S, Szigeti G, Yaguchi T, Frisvad JC (2014). Phylogeny, identification and nomenclature of the genus Aspergillus. Studies in Mycology 78:141-173.

Seetha A, Munthali W, Msere HW, Swai E, Muzanila Y, Sichone E, Okori P (2017). Occurrence of aflatoxins and its management in diverse cropping systems of central Tanzania. Mycotoxin Research 33(4):323-331.

Shabani I, Kimanya ME, Gichuhi PN, Bonsi C, Bovell-Benjamin AC (2015). Maize storage and consumption practices of farmers in Handeni District, Tanzania: Corollaries for Mycotoxin Contamination. Open Journal of Preventive Medicine 5(08):330-339.

Shirima CP, Kimanya ME, Routledge MN, Srey C, Kinabo JL, Humpf HU, Wild CP, Tu YK, Gong YY (2015). A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. Environmental Health Perspectives 123(2):173-178.

Toma A (2019). Knowledge, attitude and practice of farmers' towards aflatoxin in cereal crops in Wolaita zone, Southern Ethiopia. East C Nutrition 14:247-254.

Udomkun P, Wossen T, Nabahungu NL, Mutegi C, Vanlauwe B, Bandyopadhyay R. (2018). Incidence and farmers' knowledge of aflatoxin contamination and control in Eastern Democratic Republic of Congo. Food Science and Nutrition 6(6):1607-1620.

United Republic of Tanzania (URT) (2016). Maize production quantity. Available at https://knoema.com/atlas/United-Republic-of Tanzania/topics/agriculture/crops-production-quantity-tonnes/maize-production.

United Republic of Tanzania (URT) (2017). Budget Speech 2017/2018. Ministry of Agriculture Livestock and Fisheries, Dar es Salaam, Tanzania 75 p.

United Republic of Tanzania (URT) (2020). World data atlas, crop production. Available at Http//www.United-Republic-of-Tanzania/topics/Agriculture/Crops-Production-Quantity-tonnes/Maize-production.

Wild CP, Baan RA, Gelderblom WC, Miller JD, Riley RT, Wu F (2012). Improving Public Health through Mycotoxin Control. (Edited by John, P.), International Agency for Research on Cancer, Lyon P 151.

World Health Organization (WHO) (2018). Aflatoxins. Food Safety Digest, Department of Food Safety and Zoonoses. World Health Organization, Geneva P 5.

Wu F, Bhatnagar D, Bui-Klimke T, Carbone I, Hellmich R, Munkvold G, Takle E (2015). Climate change impacts on mycotoxin risks in US maize. World Mycotoxin Journal 4(1):79-93.

Questionnaire for Smallholder – Farmers

A. General information

1. Date ...../...../.....
2. Place (i) District..... (ii)Ward……..…… (iii)Village…..……
3. Age of respondent …….....
4. Sex of respondent……....
5. Occupation…..

6. Current education level

i) Primary Education ( ) iv) Secondary education ( )
ii) Not educated ( ) v) Tertiary education ( )
iii) University ( )

7. Marital status

i) Single ( ) iii) Married ( )
ii) Divorced ( ) iv) Widowed ( )

B. Occurrence of molds and aflatoxins contamination in foods.

1.	Have you ever heard of a mould toxin that may be present in crops? (Y/N)
2.	Have you ever heard of a mould toxin that may be present in food? (Y/N)
3.	Have you ever heard about aflatoxin? (Y/N)
4.	Are you aware that aflatoxin can contaminate crops on farm? (Y/N)
5.	Are you aware that aflatoxin can contaminate crops in storage? (Y/N)
6.	Are you aware that aflatoxin can contaminate food? (Y/N)
7.	Are you aware that Aflatoxins can be transferred to animals? (Y/N)
8.	Are you aware that Aflatoxins can be transferred into milk and dairy products?
9.	Are you aware that Aflatoxins can be transferred into breast milk? (Y/N)
10.	Are you aware of aflatoxins contamination? in crops in the field and during storage? (Y/N)
	C. Cause of aflatoxins contamination
1.	Aflatoxins can be caused by fungi? (Y/N)
2.	Aflatoxins can be caused by high levels of rain during harvesting? (Y/N)
3.	Aware that fungi infect food when stored in moist conditions? (Y/N)
4.	Aflatoxins can be caused by delayed harvesting? (Y/N)
5.	Aflatoxins can be caused by delayed drying? (Y/N)
6.	Aflatoxins can be caused by Insect infestation? (Y/N)
7.	Broken and bruised crops increase a chance of contaminations?(Y/N)
8.	Crops which contain foreign materials promote aflatoxins?(Y/N)
9.	Poor storage conditions promote aflatoxins contamination in crops ?(Y/N)
	D. Effect of aflatoxins contaminations
1.	Aflatoxins contamination reduces animal productivity? (Y/N)
2.	Aflatoxins contamination causes stunting in animals? (Y/N)
3.	Aflatoxins contamination causes death in animals? (Y/N)
	F. Health effect associated with consumption contaminated food
1.	Are you aware of the harmful effects of aflatoxins on humans? (Y/N)
2.	Are you aware the effects of aflatoxins on animals? (Y/N)
3.	Some liver diseases have been linked to intake of aflatoxins?
4.	Aflatoxins cause cancer in humans? (Y/N)
5.	Aflatoxins delay child growth? (Y/N)
6.	Aflatoxin contamination can reduce the price of crops? (Y/N)

Questionnaire for Consumer

A. General information

1. Date ...../...../.....
2. Place (i) Region..... (ii) District..... (iii)Ward…… (iv)Village……
3. Age of respondent ……...
4. Sex of respondent……
5. Occupation….....
6. Current education level

i) Primary Education ( ) iv) Secondary Education ( )
ii) Not educated ( ) v) Tertiary Education ( )
iii) University ( )

7. Marital status

i) Single ( ) iii) Married ( )
ii) Divorced ( ) iv) Separated ( )
iii) Widowed ( )

B. Occurrence of molds and aflatoxins contamination in foods.

1.	Have you ever heard of a mould toxin that may be present in crops? (Y/N)
2.	Have you ever heard of a mould toxin that may be present in food? (Y/N)
3.	Have you ever heard about aflatoxin? (Y/N)
4.	Are you aware that aflatoxin can contaminate crops on farm? (Y/N)
5.	Are you aware that aflatoxin can contaminate crops in storage? (Y/N)
6.	Are you aware that aflatoxin can contaminate food? (Y/N)
7.	Are you aware that Aflatoxins can be transferred to animals? (Y/N)
8.	Are you aware that Aflatoxins can be transferred into milk and dairy products?
9.	Are you aware that Aflatoxins can be transferred into breast milk? (Y/N)
10.	Are you aware of aflatoxins contamination? in crops in the field and during storage? (Y/N)
	C. Cause of aflatoxins contamination
1.	Aflatoxins can be caused by fungi? (Y/N)
2.	Aflatoxins can be caused by high levels of rain during harvesting? (Y/N)
3.	Aware that fungi infect food when stored in moist conditions? (Y/N)
4.	Aflatoxins can be caused by delayed harvesting? (Y/N)
5.	Aflatoxins can be caused by delayed drying? (Y/N)
6.	Aflatoxins can be caused by Insect infestation? (Y/N)
7.	Broken and bruised crops increase a chance of contaminations?(Y/N)
8.	Crops which contain foreign materials promote aflatoxins?(Y/N)
9.	Poor storage conditions promote aflatoxins contamination in crops ?(Y/N)
	D. Effect of aflatoxins contaminations
1.	Aflatoxins contamination reduces animal productivity? (Y/N)
2.	Aflatoxins contamination causes stunting in animals? (Y/N)
3.	Aflatoxins contamination causes death in animals? (Y/N)
	F. Health effect associated with consumption contaminated food
1.	Are you aware of the harmful effects of aflatoxins on humans? (Y/N)
2.	Are you aware the effects of aflatoxins on animals? (Y/N)
3.	Some liver diseases have been linked to intake of aflatoxins?
4.	Aflatoxins cause cancer in humans? (Y/N)
5.	Aflatoxins delay child growth? (Y/N)
6.	Aflatoxin contamination can reduce the price of crops? (Y/N)

Open structured questionnaire for Traders

A. General information

a. Date ...../...../.....
b. Place (i) District..... (ii)Ward…… (iii)Village....
c. Age of respondent ……....
d. Sex of respondent……
e. Occupation….....
f. Current education level
i) Primary education ( ) iv) Secondary education ( )
ii) Not educated ( ) V) Tertiary education ( )
iii) University ( )
g. Marital status
i) Single ( ) iii) Married ( )
ii) Divorced ( ) iv) Separated ( )
iii) Widowed ( )

B Postharvest handling practices

1) Which crop do you sell?
a) Maize ( )
b) Others (please mention).....
2) How do you keep your maize after buying?
a) Bare ground ( ) d) Raised platforms ( )
b) Tarpaulin ( ) e) Jute/Sisal bags ( )
c) Plastic/synthetic bags ( ) f) others (specify) ……..
3) How do you transport your maize after buying?
a) Bicycle ( ) d) Open vehicle ( )
b) Closed vehicles ( ) e) Head ( )
c) Others (Please specify)……..
4) What action do you take if it rains while your maize is at an open space?
a) Cover ( ) c) Take to the protected area ( )
b) Not cover ( ) d) others ……
5) Do you sort or clean grains before storage? (Yes/ No).....
6) If yes, how do you sort?
a) By separating from coloured grain ( ) c)Separate damage/broken grain ( )
b) By separating rotten grain ( ) d) other.....
7) What type of storage/facility do you use to store your maize?
a) Bins /Silo ( ) d) Jute/Sisal bags ( )
b) Plastic/synthetic bags ( ) e) Granaries ( )
c) Others (Please specify) ……..……..
8) How long do you store your maize before selling? …… (months)
9) How do you store your maize?
a) As cobs ( ) c) As grain ( )
b) As pods ( ) d) others (Please specify) ……..
10) Do you fumigate storehouse/warehouse before storing your maize? (Yes/No).....
11) Which of the following losses do you encounter?
a) Insect and rats infestation (Yes/No).....,
b) Mouldy/rotting (Yes/ No).....
c) Mechanical damage of grains (Yes/No).....
d) Loss of grains during shelling, storage and transport (Yes/No).....
e) Others (Please specify) ……

12) Do you use pesticides to store your maize? (Yes/No).....

B. Occurrence of molds and aflatoxins contamination in foods.

1.	Have you ever heard of a mould toxin that may be present in crops? (Y/N)
2.	Have you ever heard of a mould toxin that may be present in food? (Y/N)
3.	Have you ever heard about aflatoxin? (Y/N)
4.	Are you aware that aflatoxin can contaminate crops on farm? (Y/N)
5.	Are you aware that aflatoxin can contaminate crops in storage? (Y/N)
6.	Are you aware that aflatoxin can contaminate food? (Y/N)
7.	Are you aware that Aflatoxins can be transferred to animals? (Y/N)
8.	Are you aware that Aflatoxins can be transferred into milk and dairy products?
9.	Are you aware that Aflatoxins can be transferred into breast milk? (Y/N)
10.	Are you aware of aflatoxins contamination? in crops in the field and during storage? (Y/N)
	C. Cause of aflatoxins contamination
1.	Aflatoxins can be caused by fungi? (Y/N)
2.	Aflatoxins can be caused by high levels of rain during harvesting? (Y/N)
3.	Aware that fungi infect food when stored in moist conditions? (Y/N)
4.	Aflatoxins can be caused by delayed harvesting? (Y/N)
5.	Aflatoxins can be caused by delayed drying? (Y/N)
6.	Aflatoxins can be caused by Insect infestation? (Y/N)
7.	Broken and bruised crops increase a chance of contaminations?(Y/N)
8.	Crops which contain foreign materials promote aflatoxins?(Y/N)
9.	Poor storage conditions promote aflatoxins contamination in crops ?(Y/N)
	D. Effect of aflatoxins contaminations
1.	Aflatoxins contamination reduces animal productivity? (Y/N)
2.	Aflatoxins contamination causes stunting in animals? (Y/N)
3.	Aflatoxins contamination causes death in animals? (Y/N)
	F. Health effect associated with consumption contaminated food
1.	Are you aware of the harmful effects of aflatoxins on humans? (Y/N)
2.	Are you aware the effects of aflatoxins on animals? (Y/N)
3.	Some liver diseases have been linked to intake of aflatoxins?
4.	Aflatoxins cause cancer in humans? (Y/N)
5.	Aflatoxins delay child growth? (Y/N)
6.	Aflatoxin contamination can reduce the price of crops? (Y/N)

Vol. 16(6), pp. 238-246, June 2022
DOI: 10.5897/AJMR2022.9644
Article Number: 21BBA3A69349
ISSN: 1996-0808
Copyright©2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Assessment of handwashing knowledge, attitude and practices among healthcare workers at Muhimbili National Hospital, Tanzania

Deus M. Mtweve and Raphael Z. Sangeda*

Department of Pharmaceutical Microbiology, Muhimbili University of Health and Allied Sciences, Tanzania.

Received 13 May, 2022; Accepted 22 June, 2022

The World Health Organization (WHO) ranks healthcare-associated infection (HCAI) as one of the top ten causes of hospital death worldwide. Hand hygiene is arguably the simplest and most effective way to prevent the transmission of HCAI between one patient to another or from patients to healthcare workers. The practical implementation of hand hygiene depends on the attitude and knowledge of health practitioners regarding hand hygiene practices. The authors, therefore, investigated the knowledge attitude and hand washing practices of healthcare workers in Tanzania. The study was an institutional-based descriptive cross-sectional study conducted at Muhimbili National Hospital between 23rd July and 21st August 2020. Ethical clearance for conducting research was issued by the Institutional Review Board of the Muhimbili University of Health and Allied Sciences, Tanzania. A total of 148 healthcare workers participated in the study. The mean age of the participants was 31.06 ± 8.160 years (range: 21 - 57). Females comprises of 50.7% of the participants. Unmarried participants constituted 61.5%. Regarding educational qualifications, 63.5% had a medical degree, while 31.8% were the nursing staff. It was found that the healthcare cadre correlated with the attitude toward hand hygiene. Of all the respondents, 62.2% had moderate knowledge about hand hygiene, while 35.10% had good knowledge. Regarding attitudes to hand hygiene practices, 62.8% had a good attitude. Concerning practices, 57.4% had good practices toward hand hygiene. More than half (64.9%) of the study participants received training in hand hygiene. Continued education and training programs should be implemented at healthcare facilities to increase hand washing compliance and knowledge among workers.

Key words: Attitude, hand hygiene, hand washing practices, HCAI, healthcare-associated infection, infection control and prevention, IPC, knowledge, Tanzania.

INTRODUCTION

Hand washing is the single most important infection prevention procedure. Washing hands with soap and water significantly reduces the number of organisms to prevent potential infections (Ahmed et al., 2020; Ejemot-

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

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

Nwadiaro et al., 2021; Freeman et al., 2014). Hand washing should be performed after arriving at work, before leaving work, between client contacts, after removing gloves, when hands are visibly soiled, before eating, after urination and defecation, after contact with body fluids, before and after performing invasive procedures, and after handling contaminated equipment (Jemal, 2018). Furthermore, the World Health Organization (WHO) introduced "my five moments for handwashing," intending to minimize healthcare-associated infection (HCAI). The five moments emphasize handwashing before touching a patient, before performing aseptic and cleaning procedures, after being at risk of exposure to body fluids, after touching a patient, and after touching the patient's surroundings (Van Nguyen et al., 2020). The time required for handwashing depends on the circumstances. High-risk areas such as nurseries usually require about a 2-min hand wash and soiled hands generally require more time (Jemal, 2018). However, the time recommended for washing hands to remove transient flora from hands range from 10 to 15 s.

HCAIs are infections that patients acquire while receiving treatment for medical or surgical conditions and are the most frequent adverse event during care delivery (Dellinger, 2016; Haque et al., 2018). HCAIs occur in all care settings, including hospitals, surgical centers, ambulatory clinics and long-term care facilities such as nursing homes and rehabilitation facilities. Globally, HCAI due to poor hand hygiene are a significant problem for the safety of the patient and the healthcare workers. HCAI impact prolonged hospital stays and increases the financial burden for patients and hospitals. It may also promote the antibiotic resistance of microorganisms due to associated treatment (Allegranzi et al., 2011).

HCAI concern 5–15% of hospitalized patients in developed countries and can affect 9–37% of those admitted to intensive care units (ICUs). Consequently, HCAI contribute to mortality and morbidity (World Health Organization, 2011).

HCAI; account for 37,000 attributable deaths in Europe and potentially many more that could be related and account for 99 000 deaths in the United States of America (Ahmed et al., 2020; World Health Organization, 2011). Despite limited data on HCAI in developing countries, the recent prevalence surveys in single hospitals in Albania, Morocco, Tunisia, and the United Republic of Tanzania indicated that HCAI prevalence rates varied between 14.8 and 19.1% (WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care, n.d.). However, the study conducted in 2002 at Kilimanjaro Christian Medical Center (KCMC) in Tanzania showed the overall prevalence of HCAI to be 14.8% (Gosling et al., 2003), and surgical site infections are as high as 40% in one medical ICU (Gosling et al., 2003; The United Republic of Tanzania, 2012).

Although there is low compliance with hand hygiene among healthcare workers in both developed and developing countries, ensuring the availability of handwashing facilities (Kaplan and McGuckin, 1986), providing regular training, and reminding healthcare workers of the importance of hand hygiene have been shown to improve compliance with hand hygiene. A study conducted in Shiraz University of Medical Sciences hospitals, 2013 - 2014, indicated that shiraz healthcare workers had proper hand hygiene knowledge and attitudes; however, compliance was rated poor (Hosseinialhashemi et al., 2015). Furthermore, the study conducted in Northeast Ethiopia highlighted that 60(65.9%) were knowledgeable and 31(34.1%) were not knowledgeable. However, most health professionals, 51(56.0%), had poor practice and 40(43.0%) had a good handwashing practice. The majority of health professionals were knowledgeable. However, they had a poor practice of handwashing (Jemal, 2018).

Identifying and understanding individual cognitive factors associated with hand hygiene may help build successful hand hygiene promotion strategies. The factors that influence behavior may include knowledge, attitudes, beliefs and personality of individuals involved. To the authors knowledge, no study in Tanzania has tried to study individual cognitive factors related to hand hygiene among healthcare workers. Our study aimed to assess knowledge attitude and handwashing practices at Muhimbili National Hospital in Tanzania. This will help address the gap and intervention needed in infection prevention control in Tanzania.

METHODOLOGY

Study designing

This study was an institutional-based, descriptive, and cross-sectional one conducted at Muhimbili National Hospital (MNH) between 23rd July and 21st August 2020. Ethical clearance for conducting research was issued by the Institutional Review Board of the Muhimbili University of Health and Allied Sciences, Tanzania. Approval for conducting research was obtained from the Teaching, Research, and Consultancy unit of the MNH. Verbal consent was obtained and participation was voluntary for all the respondents.

Participants

Research participants were medical students, medical doctors, nurses, pharmacists, specialists from the obstetrics and gynecology department, surgery department, internal medicine department, and pediatrics department.

Sampling

A simple random sampling technique was used to select the study participants. A sample size of 148 was required to obtain a confidence level of 95% with a confidential interval of 5%. The sample size calculation was based on the research conducted in Pakistan, where the general knowledge level was 87.3% (Rao et

al., 2012). The online survey tool REDcap was used in data collection. Healthcare workers were interviewed and then responses were recorded (Harris et al., 2009, 2019).

Questionnaire designing and scoring

The questionnaire was a modified version of the standardized questionnaire of WHO. A pilot survey was conducted using this modified version of the questionnaire and internal consistency was tested before its application to the designated sample (WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care, n.d.; World Health Organization, 2009a, 2009b). The questionnaire included participants' social demographic characteristics, hand hygiene knowledge, hand washing practices, and attitude toward hand hygiene.

The demographics included age, gender, education level, profession, work experience, department of practices, and if they received handwashing training.

The knowledge questions were adopted from the WHO hand hygiene knowledge questionnaire and others previously used (Nair et al., 2014; World Health Organization, 2009a). Fourteen questions were asked where correct answer = 1 and wrong answer = 0. The attitude and handwashing questions were sampled from the WHO perception questionnaire and previously published research (World Health Organization, 2009b). Likert scale of 5 points were 1 = strongly agree, 2 = agree, 3 = neutral, 4 = strongly disagree and 5 = strongly disagree were used. The score in each part was high (greater or equal to 75%), moderate (74% - 50%) and poor (less than 50%). The score was adopted from research conducted in the Tertiary healthcare Centre in Raichur, India (Nair et al., 2014) and categorized as high hand hygiene, knowledge, high hand washing practices, and high attitude towards hand hygiene.

Data availability statement

The dataset associated with this study is privately stored at DRYAD repository https://datadryad.org/stash/share/r3nE_EHOtZo_vqFM_8NRci6YyUbNxOvYUFHwfYsrye8 With doi 10.5061/dryad.kd51c5b6q.

Statistical analysis

Data were extracted from Research Electronic Data Capture (REDCap) and then exported to Statistical Package for the Social Sciences (SPSS) version 24 for data analysis. The collected data were subjected to data quality scrutiny and cleaning. The results were presented using frequency, tables and charts. A Chi-square or Fisher-exact tests were used to test associations between categorical variables with knowledge attitude and hand hygiene practices. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Out of 173 healthcare workers who were approached for consent to participate in the study, only 169 (97.7%) agreed to this survey, while 4 (2.3%) rejected participating in this survey. Of those accepted to participate, 148 (87.6%) had complete responses used for data analysis. In contrast, 21 (12.4%) had incomplete responses, therefore omitted from the data analysis.

Social demographics data

Of 148 respondents, the majority (50.7%) was female and 61.5% were single. In terms of religious denominations Christians predominated at the proportion of 77%. Most (63.5%) of the participants were bachelor's degree holders. The professional cadre of nurses constituted 31.8% of the respondents. The majority of participants (41.9%) were from the obstetrics and gynecology department (Table 1). A high proportion of the participants (64.9%) received formal training in hand hygiene practices.

Knowledge of healthcare workers toward hand washing

Out of 148 healthcare workers enrolled in the study, most healthcare workers (62.2%) had moderate knowledge (Figure 1). Among all healthcare workers, 72 (48.7%) did not know the most critical reason healthcare workers practice good hand hygiene, 82 (55.4%) did not know the main route of cross-transmission of potentially harmful germs between patients in a healthcare facility. A total of 82 (58.1%) knew that alcohol-based hand rub is the best agent for killing bacteria. It was found that 124 (83.8%) agree that the healthcare worker's hands are a source for spreading resistant organisms to other patients.

Handwashing practices among healthcare workers at Muhimbili National Hospital

Of all 148 respondents, 85 (57.4%) were categorized as having good hand hygiene practices, 58 (39.2%) were categorized as having moderate hand hygiene and also 5 (3.4%) were categorized as having poor hand hygiene practices (Figure 2).

Healthcare workers' hand hygiene practices according to five moments of handwashing, 102 (68.9%) washed their hands before touching a patient, 130 (87.6%) washed their hands before an aseptic procedure, 139 (93.9%) washed their hands after being exposed to body fluids of a patient 122 (82.4%) washed their hands after touching patient 87 (58.8%) washed their hands after touching patient's surroundings (Table 2).

Attitude towards handwashing among healthcare workers

Out of 148 respondents, the majority (62.8%) were categorized as having a good attitude towards hand washing (Figure 3). (50%) considered hand washing practices to be useful, (54%) answered that it is not difficult to perform hand hygiene, (85.8%) perceived education on hand hygiene to each healthcare worker would improve hand hygiene permanently in your institution, (89.2%) agreed if leaders and senior managers

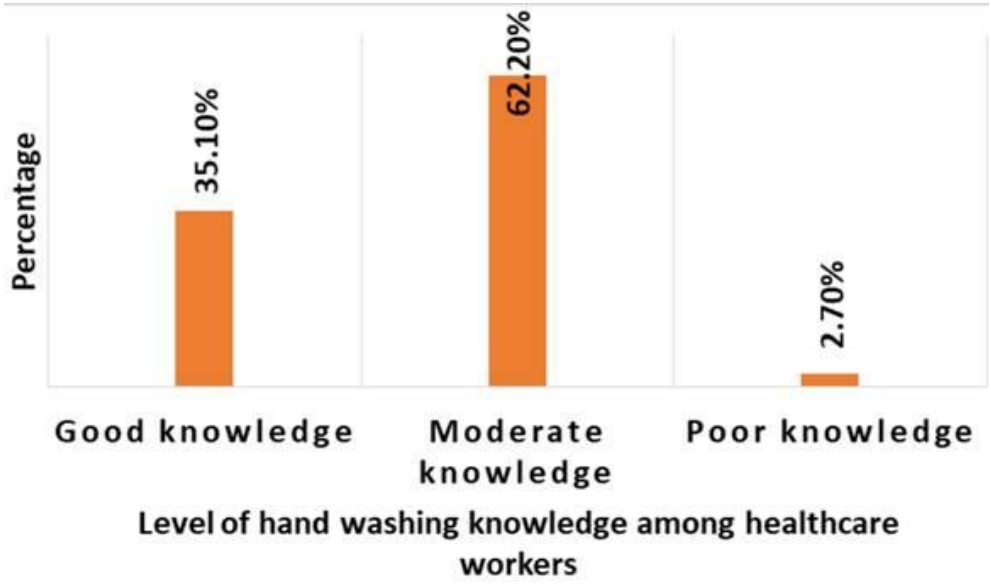

Figure 1. Level of knowledge among healthcare workers at Muhimbili National Hospital (N = 148).
Source: Authors

Figure 2. Level of hand hygiene practices among healthcare workers at Muhimbili National Hospital (N = 148).
Source: Authors

at Muhimbili National Hospital support and openly promote hand hygiene would improve hand hygiene.

Association between socio-demographic characteristics with hand hygiene practices and attitude

There was no statistically significant difference in healthcare workers' knowledge, attitude, and handwashing practices between gender, marital status, education level, and professional experience (Table 3). However, there were statistically significant observed in the profession's attitude toward hand hygiene.

DISCUSSION

There is extensive evidence of knowledge on how proper hand hygiene practices can avoid the problem of HCAI in many healthcare settings (Ahmed et al., 2020). The authors therefore, conducted a study to investigate the

Table 1. Socio-demographic characteristics of healthcare workers at Muhimbili National Hospital (N = 148).

Variable	Frequency (%)
Gender	
Female	75 (50.7)
Male	73 (49.3)
Marital status	
Single	91 (61.5)
Married	52 (35.1)
Widowed	5 (3.4)
Religion	
Christian	114 (77.0)
Muslim	33 (22.3)
Other	1 (0.7)
Education level	
Medical students	26 (17.6)
Certificate	5 (3.4)
Diploma	20 (13.5)
Degree	94 (63.5)
Others	3 (2)
Profession Cadre	
Medical doctor	44 (29.7)
Pharmacist	29 (19.6)
Nurses	47 (31.8)
Medical student	24 (16.2)
Others	4 (2.7)
Formal hand hygiene training	
Yes	96 (64.9)
No	52 (35.1)
Department	
Obstetrics and gynecology	62 (41.9)
Surgery	36 (24.3)
Internal medicine	42 (28.4)
Pediatrics	2 (1.4)
Others	6 (4.1)

Source: Authors

knowledge, attitude and practice of hand hygiene in Tanzania, to add up to this body of knowledge.

A total of 148 healthcare workers from the cadres of doctors, nurses, pharmacists and medical students consented to participate in this survey. A high proportion (62.2%) of respondents showed moderate knowledge. In addition, 57.4% had good hand hygiene practices, while also (62.8%) had a good attitude toward hand hygiene.

Among all participants, 62.2% of MNH healthcare workers had moderate knowledge. This is low compared to research conducted in two teaching hospitals (Hashemi-Nejad and Emem Reza hospitals) in Mashhad, Iran, between May 2014 and September 2015 (Zakeri et al., 2017). The majority (68%) of respondents had moderate knowledge. On another occasion, a study conducted among medical residents in Imam Hossein hospital, Iran, in 2013 showed that medical residents had moderate knowledge of hand hygiene, 65.7%, higher

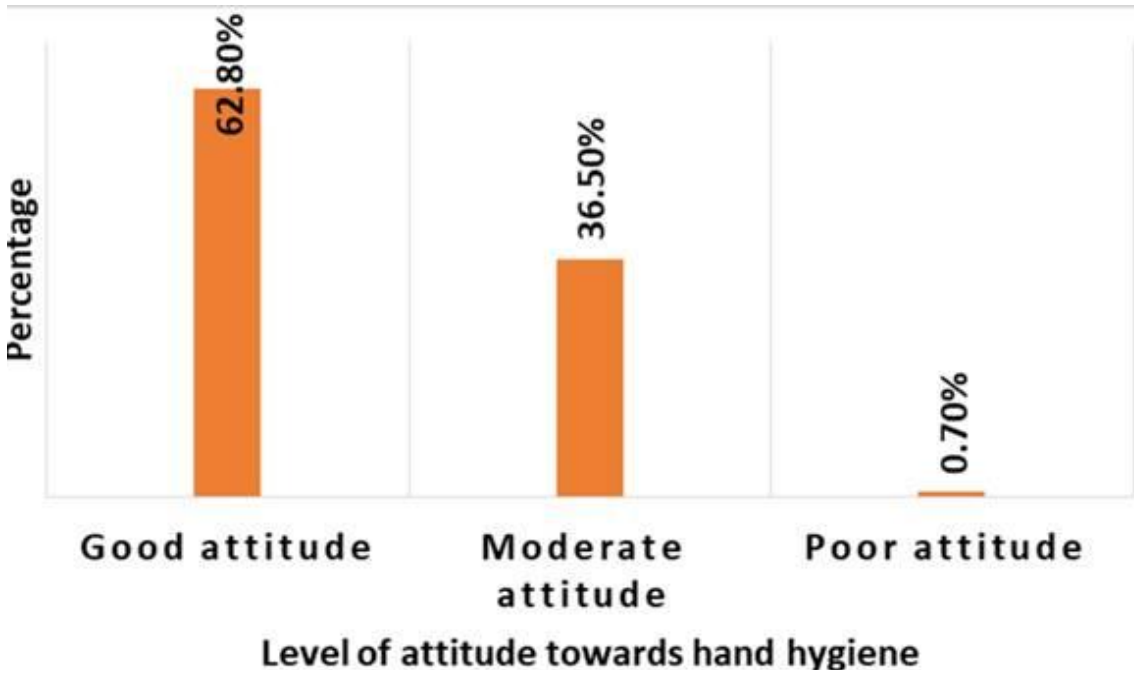

Figure 3. Level of attitude towards hand hygiene among healthcare workers at Muhimbili National Hospital (N = 148).
Source: Authors

Table 2. Responses to the five moments of handwashing practices among healthcare workers at MNH.

Moments of hand hygiene	Yes (%)	No (%)	I Don't Know (%)
Before touching patient	102 (68.9)	25 (16.9)	21 (14.2)
Before clean/aseptic procedure	130 (87.8)	3 (2)	15 (10.1)
After body fluid exposure risk	139 (93.9)	5 (3.4)	4 (2.7)
After touching patient	122 (82.4)	11 (7.4)	15 (10.1)
After touching patient surroundings	87 (58.8)	61 (41.2)	0 (0)

Source: Authors

than the findings (Nabavi et al., 2015). The results point to an increased need to improve hand hygiene conditions and educate them further according to the WHO guidelines. It was note that, despite nurses spending much time with patients because of their work schedule, their hand hygiene knowledge is as good as expected. Findings showed that (76.6%) of nurses had moderate hand hygiene knowledge and (23.4%) had good knowledge. This underscores the need for increased training and emphasis on hand hygiene among nurses. Healthcare workers' characteristics had no significant effect on hand hygiene knowledge. Of all participants (51.4%) knew the single most important reason for healthcare workers to practice good hand hygiene. A cross-sectional, hospital-based survey conducted in major public sector hospitals of Faisalabad, Lahore, Quetta, Islamabad Multan, Jamshoro and Peshawar showed that healthcare workers had good (98%) knowledge. Concerning the reason for healthcare workers practice hand hygiene (Rao et al., 2012), 44.6% knew the main route of cross-transmission of potentially harmful germs between patients in a healthcare facility, (58.1%) knew that alcohol-based hand rub is the best agent in killing bacteria (18.2%), knew how much time would an ICU nurse save during an 8-h shift by using an alcohol-based hand rub instead of soap and water. This explains the importance of increasing training among healthcare workers to improve hand hygiene knowledge in essential areas.

Of the respondents in the study, 57.4% scored good handwashing practices. Regarding five handwashing moments, 130 (87.8%) participants always washed their

Table 3. Association between healthcare worker's characteristics and attitude toward hand hygiene.

Variable		Hand washing practices N (%)			p-value
		Good (%)	Moderate (%)	Poor (%)	
Gender	Male	44 (60.3)	28 (38.4)	1 (1.4)	0.610
	Female	49 (65.3)	45 (34.7)	0 (0.0)	
Marital status	Single	57 (62.6)	34 (37.4)	0 (0)	0.190
	Married	31 (59.6)	20 (38.5)	1 (1.9)	
	Widowed	5 (100)	0 (0.0)	0 (0)	
Education level	Medical students	15 (57.7)	11 (42.3)	0 (0)	
	Certificate	4 (80)	1 (20)	0	
	Diploma	14 (70.0)	6 (30)	0 (0)	0.706
	Degree	57 (60.6)	36 (38.3)	1 (1.1)	
	Others	3 (100)	0 (0)	0 (0)	
Profession	Medical doctor	18 (40.9)	25 (56.8)	1 (2.3)	**0.03**
	Pharmacist	25 (86.2)	4 (13.8)	0 (0)	
	Nurse	33 (70.2)	14 (29.8)	0 (0)	
	Medical students	15 (62.5)	9 (37.5)	0 (0)	
	Others	2 (50)	2 (50)		
Professional experiences	0 - 10	77 (0)	42 (59.3)	0 (0)	0.213
	11 - 20	11 (50)	10 (45.5)	1 (4.5)	
	21 - 30	3 (60)	2 (40)	0 (0)	
	31 - 40	2 (100)	0 (0)	0 (0)	
Age groups	18 - 29	56 (65.1)	30 (34.9)	0 (0)	0.342
	30 - 39	21 (63.6)	12 (36.4)	0 (0)	
	40 - 49	13 (52)	11 (44)	1 (4)	
	50 +	3 (75)	3 (25)	0 (0)	

Source: Authors

hands before clean and aseptic procedures. The result was higher than the results obtained in Northeast Ethiopia, where 36.3% of participants washed their hands before clean and aseptic procedures (Jemal, 2018). About 102 (68.9%) of participants always washed their hands before individual patient contact, but this result was higher compared to 21 (60.1%) in a study conducted in Pakistan. In addition, 64 (43.2%) always used alcohol-based hand rub for hand hygiene.

In the current study, only 122 (82.4%) washed their hands after contact with patients, compared to a survey conducted in Ethiopia, where 78% of healthcare workers washed their hands after contact with body secretions. At the same time, research showed that 139 (93.9%) washed hands after contact with body secretions. When comparing these two studies, healthcare workers adhere to washing hands after body fluid exposure more often than the other five moments of hand hygiene. This can be explained that healthcare workers are more concerned about conditions threatening their health than conditions threatening patients' health. Therefore, healthcare workers' major concern was to protect themselves.

Healthcare workers' characteristics showed no statistically significant relationship with handwashing practices. However, the study done in Australia showed that gender played an important role in influencing healthcare workers' handwashing rate (van de Mortel et al., 2001).

In this study, only 93 (62.8%) were categorized as having a good attitude towards hand hygiene. However, the survey conducted in Jordan showed that attitude towards handwashing was 65.28% higher than the results (Ghafari and Aburuz, 2019).

It was lower than the overall attitude towards hand hygiene conducted at Anuradhapura Teaching Hospital Sri Lanka, whereby 47.5% had good attitudes, 42.5% had

moderate attitudes and 10% showed poor hand hygiene attitudes (Kudavidnange et al., 2013). Most studied healthcare workers had a positive attitude toward hand hygiene in the present study. However, 50% strongly agreed that hand hygiene is helpful before and after touching the patient, before clean/ aseptic procedures, after body fluid exposure, after touching the patient, and after touching the patient's surroundings.

Moreover, 28% agreed that it is difficult to comply with hand hygiene to improve hand hygiene compliance. Of all participants, 81.7% agreed that if healthcare facilities make alcohol-based hand rub always available, it will improve hand hygiene permanently in their institution. Furthermore, the majority of healthcare workers believed that displaying reminders, education and promotion of hand hygiene by seniors and leaders would improve hand hygiene practices in their institution.

Some study constraints may limit the interpretation of the results. First, this was only a single-center study conducted at the National Hospital in the country. The current setting may benefit from more availability of knowledgeable healthcare workers in aspects of hand hygiene as opposed to remote settings in the country. Secondly, because the study was conducted during the first peak of the emerging COVID-19 pandemic, most responses may have been influenced by the prevailing pandemic. In addition, the proportion of different cadres may not be well balanced, and at the time of the survey, a few medical students in their final years of training were included in the study. The inclusion of these medical students may have counteracted some of the findings. Nevertheless, this cadre of healthcare workers has a pivotal role in serving the patients at the study site. Therefore, their responses were relevant to the practice of hand hygiene in Tanzania.

Conclusion

Even though most healthcare workers were found to have good hand washing practices and attitudes, there is an urgent need to introduce measures and strategies to increase the knowledge, attitudes and handwashing practices at Muhimbili National Hospital and other healthcare settings in Tanzania. The suggested initiative may play a crucial role in improving hand hygiene compliance among healthcare workers. Finally, it was recommend that further study should be conducted to observe handwashing practices and assess the effect of availability of handwashing facilities on handwashing practices, knowledge and attitude toward hand hygiene among healthcare workers.

ACKNOWLEDGMENT

The authors would like to thank all the participating healthcare workers.

ETHICS APPROVAL

Consent was obtained or waived by all participants in this study. Muhimbili University of Health and Allied Sciences issued approval DA.25/111/01/10/Feb/2020. Ethical clearance for conducting research was issued by the Institutional Review Board of the Muhimbili University of Health and Allied Sciences, Tanzania. Approval for conducting research was obtained from the Teaching, Research, and Consultancy unit of the Muhimbili National Hospital. Verbal consent was obtained and participation was voluntary for all the respondents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmed J, Malik F, Memon Z A, Bin Arif T, Ali A, Nasim S, Ahmad J, Khan M A (2020). Compliance and Knowledge of Healthcare Workers Regarding Hand Hygiene and Use of Disinfectants: A Study Based in Karachi. Cureus 12(2).

Allegranzi B, Nejad SB, Combescure C, Graafmans W, Attar H, Donaldson L, Pittet D (2011). Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. The Lancet 377(9761):228-241.

Dellinger EP (2016). Prevention of Hospital-Acquired Infections. Surgical Infections *17(4):422*-426.

Ejemot-Nwadiaro RI, Ehiri JE, Arikpo D, Meremikwu MM, Critchley JA (2021). Hand-washing promotion for preventing diarrhoea. *Cochrane* Database of Systematic Reviews 1.

Freeman MC, Stocks ME, Cumming O, Jeandron A, Higgins JPT, Wolf J, Prüss-Ustün A, Bonjour S, Hunter PR, Fewtrell L, Curtis V (2014). Hygiene and health: systematic review of handwashing practices worldwide and update of health effects. Tropical Medicine and International Health 19(8):906-916.

Ghafari ZA, Aburuz ME (2019). Hand Hygiene Knowledge , Attitude and Barriers among Jordanian Nurses. International Medical Journal, 24(03):385-400.

Gosling R, Mbatia R, Savage A, Mulligan JA, Reyburn H (2003). Prevalence of hospital-acquired infections in a tertiary referral hospital in northern Tanzania. Annals of Tropical Medicine and Parasitology 97(1):69-73.

Haque M, Sartelli M, McKimm J, Abu Bakar M (2018). Health care-associated infections - an overview. Infection and Drug Resistance 11:2321-2333.

Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, Duda SN (2019). The REDCap consortium: Building an international community of software platform partners. Journal of Biomedical Informatics 95:103208.

Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG (2009). Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. Journal of Biomedical Informatics 42(2):377-381.

Hosseinialhashemi M, Kermani FS, Palenik CJ, Pourasghari H, Askarian M (2015). Knowledge, attitudes, and practices of health care personnel concerning hand hygiene in Shiraz University of Medical Sciences hospitals, 2013-2014. American Journal of Infection Control 43(9):1009-1011.

Jemal S (2018). Knowledge and Practices of Hand Washing among Health Professionals in Dubti Referral Hospital, Dubti, Afar, Northeast Ethiopia. Advances in Preventive Medicine 1-7.

Kaplan LM, McGuckin M (1986). Increasing handwashing compliance with more accessible sinks. Infection Control 7(8):408-410.

Kudavidnange B, Gunasekara T, Hapuarachchi S (2013). Knowledge, attitudes and practices on hand hygiene among ICU staff in Anuradhapura Teaching hospital. Anuradhapura Medical Journal 5(1):29.

Nabavi M, Alavi-Moghaddam M, Gachkar L, Moeinian M (2015). Knowledge, Attitudes, and Practices Study on Hand Hygiene Among Imam Hossein Hospital's Residents in 2013. Iranian Red Crescent Medical Journal 17(10).

Nair SS, Hanumantappa R, Hiremath SG, Siraj MA, Raghunath P (2014). Knowledge, Attitude, and Practice of Hand Hygiene among Medical and Nursing Students at a Tertiary Health Care Centre in Raichur, India. International Scholarly Research Notices Preventive Medicine pp. 1-4.

Rao MH, Arain GM, Khan MI, Taseer I, Talreja K, Ali G, Munir MK, Naz S, Hussain I, Ahmed J, Talreja L, Ali G, Munir MK, Naz S, Hussain I, Ahmed J (2012). Assessment of Knowledge, Attitude and Practices Pattern of Hand Washing in Some Major Public Sector Hospitals of Pakistan (A Multi-Center Study). Pakistan Journal of Medical Research 51(3):76-82.

The United Republic of Tanzania (2012). *National Communication Strategy for Infection Prevention and Control 2012-2017*. http://www.tzdpg.or.tz/fileadmin/documents/dpg_internal/dpg_working_groups_clusters/cluster_2/health/Sub_Sector_Group/Quality_Assurance/07_IPC_Communication_Strategy_FINAL.pdf

van de Mortel T, Bourke R, McLoughlin J, Nonu M, Reis M (2001). Gender influences handwashing rates in the critical care unit. American Journal of Infection Control 29(6):395-399.

Van Nguyen H, Tran HT, Khuong LQ, Van Nguyen T, Ho NT, Dao AT, Van Hoang M (2020). Healthcare Workers' Knowledge and Attitudes Regarding the World Health Organization's "My 5 Moments for Hand Hygiene": Evidence From a Vietnamese Central General Hospital. Journal of Preventive Medicine and Public Health 53(4):236-244.

WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care (2009). Retrieved May 2, 2022, from https://www.ncbi.nlm.nih.gov/books/NBK144030/

World Health Organization (2011). Report on the burden of endemic health care-associated infection worldwide*: Clean care is safer care*. https://apps.who.int/iris/bitstream/handle/10665/80135/9789241501507_eng.pdf?sequence=1

World Health Organization (2009a). Hand Hygiene Knowledge Questionnaire for Health-Care Workers. https://cdn.who.int/media/docs/default-source/integrated-health-services-(ihs)/hand-hygiene/monitoring/surveyform/hand-hygiene-knowledge-questionnaire.doc?sfvrsn=dbb4da65_2

World Health Organization (2009b). Perception Survey for Health-Care Workers. Available online: https://cdn.who.int/media/docs/default-source/integrated-health-services-(ihs)/hand-hygiene/monitoring/surveyform/perception-survey-for-health-care-workers.doc?sfvrsn=8fa7cb79_2

Zakeri H, Ahmadi F, Rafeemanesh E, Afshari Saleh L (2017). The knowledge of hand hygiene among the healthcare workers of two teaching hospitals in Mashhad. Electronic Physician 9(8):5159-5165.

Vol. 16(6), pp. 247-257, June 2022
DOI: 10.5897/AJMR2020.9341
Article Number: 3389F5769392
ISSN: 1996-0808
Copyright©2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Biodegradability of polystyrene plastics by bacterial isolates from plastic composted waste soil and molecular characterization of plastic degrading bacterial isolates

Ugueri Udochukwu[1,2]*, E. I. Atuanya[2] and Zainab Usman[3]

[1]Department of Biosciences, Salem University, Lokoja, Kogi State, Nigeria.
[2]Department of Microbiology, University of Benin, Edo State, Nigeria.
[3]Department of Science Labouratory, Kogi State Polytechnic, Kogi State, Nigeria.

Received 17 April, 2020; Accepted 19 June, 2020

This study examined the biodegradability of polystyrene (PS) plastics. Soil samples were collected from Oluku Community in Egor Local Government Area, Benin City, Edo State, Nigeria. Heterotrophic bacteria were enumerated and screened for PS degradation potential. Plastics degrading potential of the isolates was determined by Shake Flask method, degradation of PS plastics was determined by analyzing the formulated PS plastic solution for its additive concentration before and after the degradation process using gas chromatograph with mass spectrometry. Identified bacterial isolates were further characterized using the 16S ribosomal RNA gene. The results from all the parameters indicate that there was active utilization of oxygen and other nutrients available in the test system which is an evidence of PS degradation. The pH had values ranging from 6.5 and 7.4. It was observed that the nutrients and the biochemical oxygen demand decreased considerably with time. There was a reduction in the concentration of bisphenol A (BPA) contingents recorded before (37.04 mg/kg) and after (1.19 mg/kg) the degradation process. The bacterial isolates with codes B1 and B3 belonging to *Bacillus* while B2 belong to *Pseudomonas* genera were identified. Two isolates had 99% similarity with *Bacillus subtilis* strain BS3902 and EU047884.1 respectively, while the third isolate had 100% similarity with *Pseudomonas aeruginosa* strain KAVKOI. This results shows that the strains have the ability and are able to degrade PS plastics.

Key words: Polystyrene plastics, plastic composted soil, biodegradability, heterotrophic bacteria, molecular characterization.

INTRODUCTION

The term “plastics” includes materials composed of various elements such as carbon, hydrogen, oxygen, nitrogen, chlorine, and sulphur. They are produced by the conversion of natural products or by the synthesis from primary chemicals generally coming from crude oil, natural gas, or coal (Coors et al., 2003; Jonsson et al.,

*Corresponding author. E-mail: rev.dr.ud@gmail.com.

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

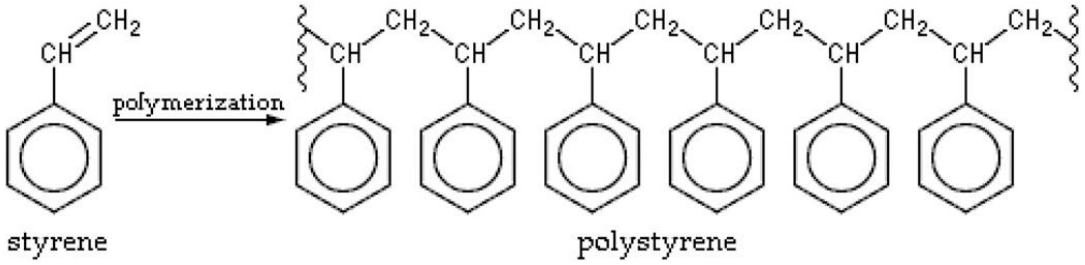

Figure 1. Chemical formula of polystyrene (Ho et al., 2018).

2003). The increased use of plastics in day to day consumer applications has resulted in municipal solid waste containing an ever growing fraction of plastic material used for a short time and discarded (Ho et al., 2018). Plastics have taken centre stage in daily life due to its qualities like low weight, durability and low cost as compared to other materials types (Andrady and Neal, 2009). Polystyrene (PS) is a synthetic aromatic polymer with high molecular weight (formula (C8H8)n) made from the monomer styrene (Figure 1) (Ho et al., 2018). Like other plastics, PS is widely used because of its good mechanical properties and relatively low cost (Ho et al., 2018). PS is widely used in construction materials (insulation), packaging foam, food containers, disposable cups, plates, cutleries, cassette boxes, and compact disks (Ho et al., 2018). There is about 21 million tons of PS produced in the world in 2013 (Yang et al., 2015). As a result of such wide use, plastics including PS have accumulated in the environment, causing environmental pollution, human health problems, and ecosystem changes due to their toxicity and recalcitrant compounds. PS materials can be recycled; however, most PS foam ends in landfill (Ho et al., 2018). Plastic pollution affects soil aeration, soil fertility, soil pH, nitrification and the activities of soil fauna and soil flora which act as sentinels in the soil (Atuanya et al., 2016).

Biodegradation of plastics is the process in which microorganisms (fungi, bacteria, and archaea) degrade them by their extracellular or intracellular enzymes and use the plastics as a substrate for growth (Adamcova and Vaverkova, 2014; Himani et al., 2013; Zheng et al., 2005). PS biodegradation starts when microorganisms begin growing on the surface of PS and secrete their enzymes to degrade the polymer into smaller molecular fragments called oligomer and maybe monomeric units (Zheng et al., 2005). Styrene itself is able to be used as a carbon source for growth by some microorganisms. *Rhodococcus ruber* has been shown to form biofilms on PS and partially degrade it (Mor and Sivan, 2008). A biofilter consisting of *Brevibacillus* species has been shown to remove 3 kg of styrene in a day (Motta et al., 2009). The biodegradation rate depends on the thickness and the molecular weight of the plastic (Hwang et al., 2008). In fact, a large number of microorganisms can bring about styrene biodegradation (Baggi et al., 1983). There are several ways of styrene catabolism; however, a predominant pathway involves the oxidation of styrene to phenylacetate, which is then converted via the TCA cycle (Mor and Sivan, 2008). This pathway is as shown in Figure 2.

Biodegradation of PS has been reported in some previous studies. In the literature, few reports describe the microbial utilization of PS as a carbon source (Kaplan et al., 1979; Sielicki et al., 1978). However, there are few reports of microbes degrading PS in the real environment such as landfill, soil, etc. Oikawa et al. (2003) isolated and identified *Pseudomonas* and *Bacillus* species for styrene degradation; also *Xanthomonas* and *Sphingobacterium* species for PS decomposition by 16 S ribosomal DNA analysis from soil (Sielicki et al., 1978). Four microbial strains have been isolated from garden soil after 8-month buried samples of PS and EPS solution (2%) in chloroform. They were identified as *Microbacterium* species NA23, *Paenibacillus urinalis* NA26, *Bacillus* spp. NB6, and *Pseudomonas aeruginosa* NB26. They were able to extract some carbon from the complex molecules of PS but the process was very slow and caused no significant chemical changes on the surface (Atiq et al., 2010). Therefore, this study examined biodegradability of polystyrene plastics by bacterial isolates from Plastic Composted waste Soil and Molecular Characterization of Plastic Degrading Bacterial Isolates.

MATERIALS AND METHODS

Sample collection

Soil samples (500 g) were collected from different locations within the waste management landfill site located at Oluku Community, Benin City, Edo State, Nigeria at a depth of 0 to 10 cm with a standard soil auger in plastic bags. The soil samples were homogenized and kept on the laboratory bench to air dry (Atuanya et al., 2012). The soil sample was used for the isolation and enumeration of total heterotrophic bacteria.

Isolation and enumeration of heterotrophic bacteria

Serial dilution of soil sample was made to form 10^{-4}, 10^{-5} and 10^{-6}

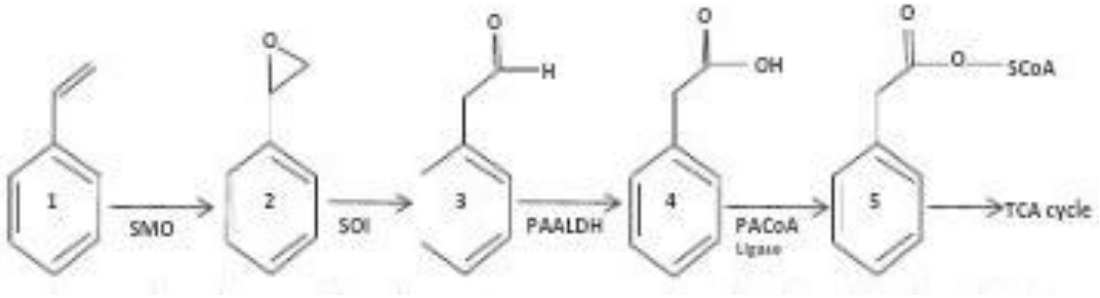

Figure 2. Degradation pathway for styrene (Tischler et al., 2009; Mooney et al., 2006). 1-styrene, 2-styrene oxide, 3-phenyl acetaldehyde, 4-phenylacetic acid, 5-phenylacetyl coenzyme A SMO: styrene monooxygenase, SOI: styrene oxide isomerase, PAALDH: phenylacetaldehyde dehydrogenase, PACoA ligase: phenylacetyl coenzyme A ligase.

dilutions using normal saline. Total viable heterotrophic bacterial counts were determined. Nutrient agar plates were prepared; the plates were inoculated and were incubated at 37°C for 24 h. Colony counts were taken after incubation and biochemical tests were carried out (Burkhard et al., 2001).

Collection and preparation of polystyrene plastic granules

Waste polystyrene plastics were collected and blended into powder using an industrial grinding machine. The plastic granules was weighed and kept in small white polyethylene bags. This polystyrene granule was used to formulate different polystyrene plastic concentration in a mineral salt medium which was used for biodegradation test (Atuanya et al., 2016).

Screening test for biodegradation potential of polystyrene plastics

Bacterial isolates were screened for the ability to degrade polystyrene plastics using mineral salt medium. 9 ml of the mineral salt medium was dispensed into seven test tubes and sterilized. In each of the test tubes, 0.1 g of plastic at 20 ppm was added to serve as the only source of carbon and energy (Atuanya et al., 2011). Thereafter, all the test tubes were inoculated with two drops of cell suspension of an isolate previously grown in mineral salt medium. The cell suspension was prepared by suspending a loopful of the bacterial isolate from nutrient agar plate into two (2 ml) mineral salt medium. Among the tubes, there was a control which was not inoculated. All the tubes were incubated at room temperature (28±2°C) for 7 days after which the tubes were checked for turbidity which indicated the ability of the isolates to utilize PS plastics as growth source (Ferrara et al., 2006).

Determination of plastics degrading potential of the isolates by shake flask method

A known volume of 150 ml of the mineral salt medium was dispensed into 250 ml conical flask and the test polystyrene (PS) plastic granules were introduced separately into the conical flask after sterilization (Nishida and Tokiwa, 1994). Overnight, broth culture of each isolate was seeded into each flask and incubated on the laboratory bench. The utilization of PS plastics was monitored at two days interval for 10 days by monitoring the bacterial growth measured by viable counts on nutrients agar. The optical density was determined at 620 nm wavelength using Comspec Visible Spectrophotometer, changes in ionic concentration and pH were determined with pH meter (Model Hanna microprocessor P211 pH meter, India) and temperature using temperature meter. Physicochemical analyses were carried out such as pH, total organic carbon, biochemical oxygen demand (BOD), alkalinity analysis, sulphate content, nitrate content and phosphate content to determine the rate of degradability of PS plastic (Brulle et al., 2010).

Determination of plastic degradation

Degradation of the PS plastic granules and the level of degradation was determined using Hewlett Packard HP 5890 series II Gas chromatograph with Mass Spectrometry before and after the degradation process.

Instrumentation and conditions

Hewlett Packard HP 5890 series II Gas chromatograph equipped with an Agilent 7683B injector (Agilent Technologies Santa Clara, CA, USA), A 30 m, 0.25 mm i.d. HP-5MS capillary column (Hewlett – Packard, Palo Alto, CA, USA) coated with 5% phenyl-methylsiloxane (film thickness 0.25 m) and an Agilent 5975 mass selective detector (MSD) was used to separate and quantify the BPA compounds. The samples were injected in the split less mode at an injection temperature of 300°C. The transfer line and ion source temperature was 280 and 200°C. The column temperature was initially held at 40°C for 1 min, raised to 120°C at the rate of 25°C/min, then to 160°C at the rate of 10°C min^{-1} and finally to 300°C at 5°C min^{-1}, held at final temperature for 15 min. Detector temperature was kept at 280°C. Helium was used as a carries gas at a constant flow rate of ml/min. Mass spectrometry was acquired using the electron ionization (EI) and selective ion monitoring (SIM) mode. A PerkinElmer Gas Chromatograph model Autosystem XL, with Flame Ionization Detector was used for identification of BPA, phthalate, organotin, alkyl phenol and other plastic components by comparison between the retention times of the BPA sample peak and the standard compound. The quantification was done by the internal normalization method. An Elite-5 fused silica capillary column (30 m × 0.25 mm i.d. crossbond 5% diphenyl 95% dimethyl polysiloxane, 0.25 µm film thickness) was used for the GC separation using the following oven temperature program: 150°C (5 min hold) heating to 250°C at 3°C min^{-1} and heating to 300°C at 10°C min^{-1} (5 min hold). The injector temperature was 250°C. The injection volume was 1.0 µL (n=3) in the split

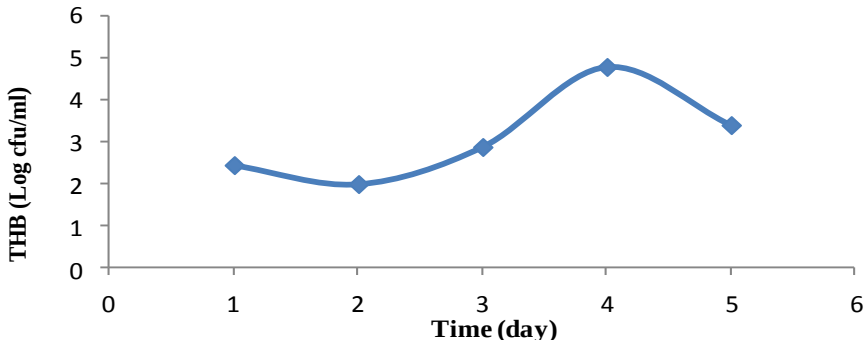

Figure 3. Change in total heterotrophic bacterial count (Log cfu/g) of the test system.

mode (1:50) (Burkhard et al., 2001).

Molecular characterization of plastic degrading bacterial isolates

DNA extraction

Bacteria in saline were added to 1.5 ml micro centrifuge tube. 450 µl of a 240 mM NaOH, 2.7 mM EDTA, and 74% ethanol solution were added to the tube and mixed gently to give final concentrations of 200 mM NaOH, 2.25 mM EDTA, 61% ethanol. The tube was then heated to 80°C for 10 min and centrifuged at 16,060 ×g for 10 min. The supernatant was removed, and 100 µL of an optimized suspension solution containing 0.1 mM EDTA, 50 Mm Tris-HCl, Ph 8.0, 1% Triton-X-100, and 0.5% Tween-20 was added to solubilize the denatured DNA. DNA was collected by centrifugation at 7200×g for 10 min, washed with 500 µl of 70% ethanol, air dried at room temperature for approximately 3 h and finally dissolved in 50 µl of TE buffer (Brosius et al., 1981).

Polymerase chain reaction procedure

The PCR consist of final volume of 50 µl which included 8 µl DNA and 42 µl reaction cocktail consisting of 5x GoTaq green reaction, 10 Mm of each dNTPs, 10 pmol each 27F: 5´-AGAGTTTGATCMTGGCTCAG-3´ and 1525 R: 5´-AAGGAGGTGWTCCARCC-3´ specific for ~ 800 bp conserved domain of the 16S rRNA polymerase. PCR was carried out using the following thermal cycles regime; an initial denaturation at 94°C for 1 min, this was followed by 29 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1 min and an extension at 72°C for 1.5 min, a final extension at 72°C for 5 min ended the PCR experiment (Brosius et al., 1981).

Agarose gel electrophoresis

Agarose gel was prepared and buffered with 1.5 ml of 0.5x TAE. 10 ml of ethidium bromide was added, mixed and then poured into electrophoretic tank with the comb in place to obtain a gel thickness of about 4 to 5 mm. 10 µl of sample was mixed with 1 µl of the 10x loading dye. DNA samples were loaded and ran. The DNA was viewed using a UV-trans-illuminator (Opere et al., 2013).

Sequencing of the 16S rDNA gene

The purified DNA samples were sequenced at the Bioscience Laboratory, International institute for tropical Agriculture (I.I.T.A), Ibadan, Oyo State with an automated DNA sequencing analyzer (ABI 3730x) using 27F and 1492R primers. Sequence assembly and alignment were carried out using CLC bio software, followed by searching the homology in the Gene Bank using Basic Local Alignment Search Tool (BLAST) program of CLC bio software.

RESULTS AND DISCUSSION

The results from this research showed evidence of polystyrene plastic degradation. All the parameters (Figures 3 to 10) indicate that there was active utilization of oxygen and other nutrients available in the test system. The pH profile obtained generally fell between the optimum range of 6.5 and 7.4 which favors most of the heterotrophic bacterial though the values did not follow a consistent trend as for the other parameters; it was observed that the metabolic products produced by PS plastic utilizing bacteria must have contributed to the fluctuation of the pH readings near neutrality. It was also observed that the nutrients (sulphate, phosphate and nitrate) decreased considerably with time. The decrease is understandable as they are used in the metabolism of microorganism in building biomass. There is correspondence in the utilization of phosphate, sulphate and nitrate indicating their relative importance in cell metabolism as stated by Odum's combine law. The biochemical oxygen demand (BOD) of the media was also decreasing as the study progressed indicating that the oxygen content in the medium is been utilized by the aerobic bacteria. There was evidence of degradation of polystyrene plastics from the concentration of Bisphenol A (BPA) contingents recorded before (37.04 mg/kg) and after (1.19 mg/kg) the degradation process shown in Table 2. Although there was no complete degradation of the polystyrene plastic, but there was a considerable reduction in the concentration of the BPA contingents, TOC, nitrate, phosphate, and sulphate in the test system (Odokuma and Okpokwasili, 1993).

There were three major plastic degrading bacterial isolates of which two were identified as *Bacillus* spp. and one as *Pseudomonas* spp. (Table 1) which was further

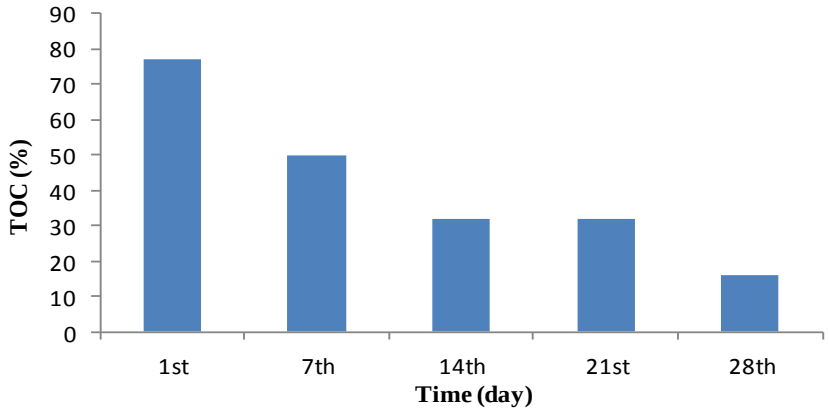

Figure 4. Change in percentage of total organic carbon of the test system.

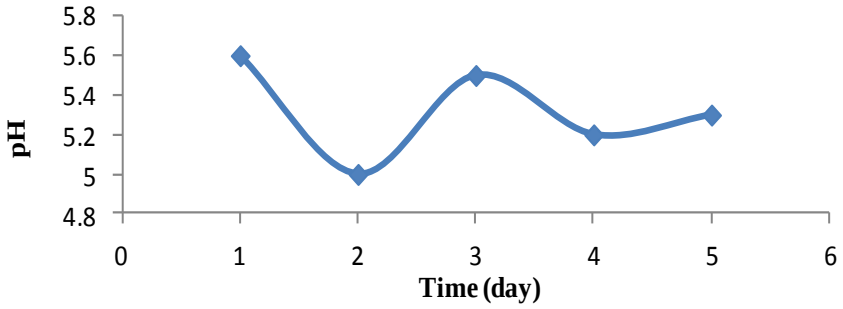

Figure 5. Change in pH of the medium for the test systems.

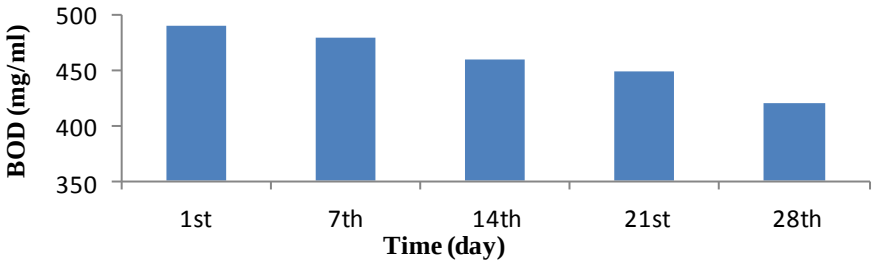

Figure 6. Change in BOD OF the test systems.

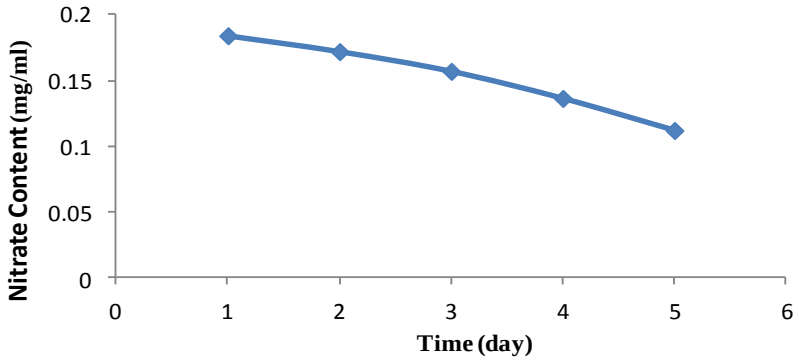

Figure 7. Change in concentration of nitrate content for the test systems.

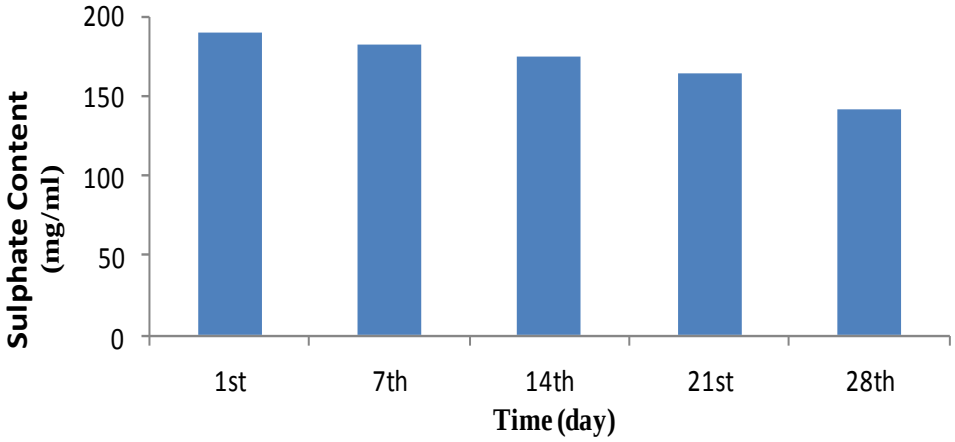

Figure 8. Change in the concentration of sulphate content of the test systems.

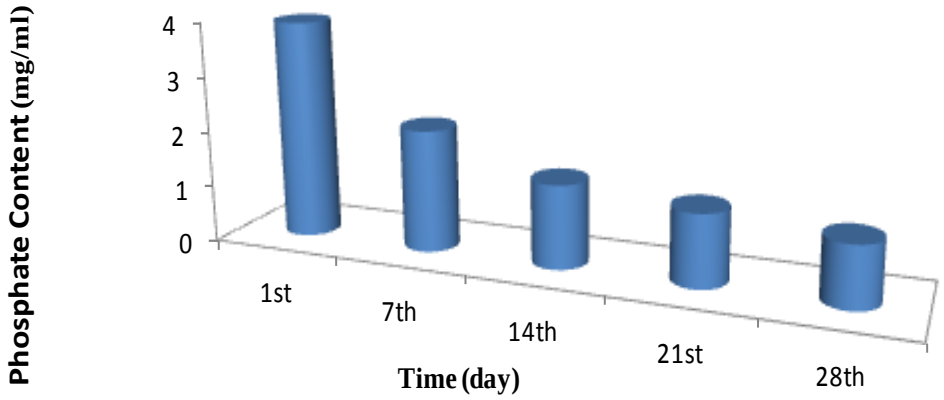

Figure 9. Change in the concentration of phosphate content of the test systems.

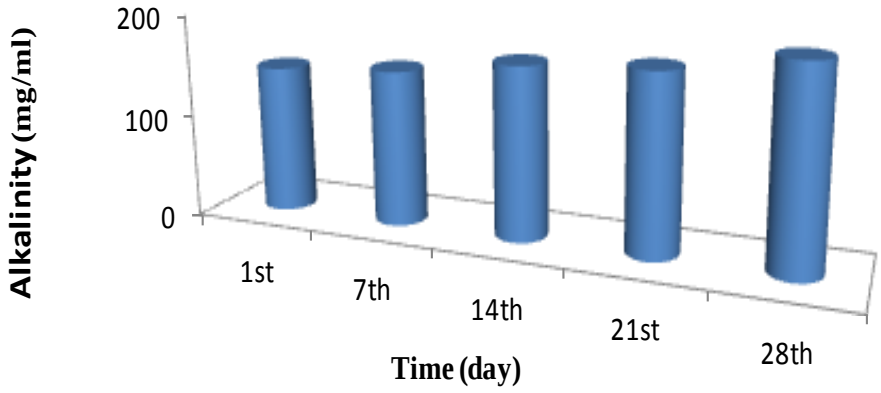

Figure 10. Change in alkalinity of the test systems.

characterized using the 16S ribosomal RNA gene (Table 3). PCR amplification using 16S rRNA gene universal primer set generated amplicons of around 500 bp fragments. This is in line with the results of previous

Table 1. Cultural, morphological and biochemical test of bacterial isolates.

Characteristics	1	2	3
Cultural			
Shape	Circular	Circular	Irregular
Elevation	Low convex	Convex	Flat
Margin	Entire	Undulated	Undulated
Wetness/dryness	Wet	Dry	Wet
Transparency	Opaque	Opaque	Opaque
Colour	Green	Cream	Cream
Size	Medium	Medium	Large
Morphological			
Gram staining	-	+	+
Cell type	Rod	Rod	Rod
Cell arrangement	Single	Chains	Large
Biochemical			
Catalase	+	+	+
Oxidase	+	-	-
Coagulase	-	-	-
Urease	-	+	+
Indole	-	-	-
Citrate	+	+	+
Sugar fermentation			
Glucose	+	+	+
Lactose	-	-	-
Possible isolates	*Pseudomonas* spp.	*Bacillus* spp.	*Bacillus* spp.

Table 2. Degradation of polystyrene plastics and the bisphenol A contingence found in the plastic composted soil sample.

Parameter	Before degradation	After degradation
Methylene	17.45	0.54
Hexane	10.05	0.26
Chloroform	1.56	0.31
Toluene	5.87	0.07
Tetrachloroethylene	1.48	0.01
Chlorobenzene	0.37	0.00
Dichlorobenzene	0.15	0.00
Benzene	0.11	0.00
Total	37.04 mg/kg	1.19 mg/kg

study as theoretically predicted for bacterial family (Opere et al., 2013). Amplicon from the first round of PCR were thereafter used as templates to run a bacterial species level, which generated PCR products of about 600 bp (Plate 1) and 550 bp (Plate 2) in size as predicted for *Bacillus* and *Pseudomonas* spp., respectively. BLAST results of the sequences obtained in this study showed an identity query coverage length of 1533, 1532 and

Table 3. 16S rRNA sequence of the plastic degrading bacterial isolates.

Isolate code	Sequence blast	Ascension no.	Sequence identity	Query coverage length	Score bits (%)
B1	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTT GCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAA CCGGGGCTAATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATG GACCCGCGGCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTG ATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACG AAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAG TACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT AATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGA AAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCAC GTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTG AGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT AGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAA CTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC AGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTG TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAG TTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATG ACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATC TGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCC GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAG GTAACCTTTTAGGAGCCAGCCGCCGAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGA AGGTGCGGCTGGATCACCTCCTT	EU047884.1	*Bacillus subtilis* strain BS3902	1533	1539/1542 (99)
B2	GGCTACACATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAG GAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGG GGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCG ACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAG CAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTA AAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGC TAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTA GGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGA CCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC CGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGG GGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTC GAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACT CAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCC TTGTCCTTAGTTACCAGCACCTCGGGTGCGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT GACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCC GCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAAT CGCTAGTAATCGTCAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGG AGTGGGTTGCTCCAGAAGTAGCTAGTCTAACCGCAAGGGG	GQ865644.1	*Pseudomonas aeruginosa* strain KAVKOI	2595	1405/1405 (100)

Table 3. Contd.

B3	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCT TGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGA AACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGA TGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGG TGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGA CGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACA AGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATG TGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTACAGAAGAGGAGAGTGGAATTC CACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGAC GCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAA GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGA CTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAA CCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGC ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCA GCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGC CCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCC CACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCA GCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGT CGGTGAGGTAACCTTTTAGGAGCCAGCCGCCAAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAGCC GTATCGGAAGGTGCGGCTGGATCACCTCCT	KR967375.1	*Bacillus subtilis* strain AER111-2	1532	1538/1541 (99)

Genome DNA of the isolates was extracted using QIAamp DNA Mini kit (250) cat no. 51306 with quagen DNA extraction protocol. Extracted DNA templates were subjected to PCR using set (Forward and Reverse) universal primers 16SF-AGAGTTTGATCMTGGCTCAG and 16SR-AAGGAGGTGWTCCARCCGCA, the primers allowed amplification of the 16Srna genes of the isolates. The base was edited with BioEditR software. The edited sequences were then used for similarity searches using Base Local Aligment Search Tool (BLAST) program in the NCBI GenBank which is a DNA database for identify bacterial strains. B1: *Bacillus subtilis* strain BS3902; B2: *Pseudomonas aeruginosa* strain KAVKOI; B3:.*Bacillus.subtilis.*strain.AER111-2.

2595. It was observed that the isolates from plastic composted soil with codes B1 and B3 belong to *Bacillus,* while B2 belong to *Pseudomonas* genera. Two isolates with accession number EU047884.1 and KR967375.1 had 99% similarity with *Bacillus subtilis* strain BS3902 and *B. subtilis* strain AER111-2, respectively, while the third isolate had 100% similarity with *P. aeruginosa* strain KAVKOI with accession number GQ865644.1. It was observed that these strains were able to degrade polystyrene plastics (Opere et al., 2013).

Polystyrene plastics have been found to be susceptible to microbial attack and hence biodegradation or even biodeterioration of these plastics can occur (Okpokwasili and Okorie, 1991). Researchers have reported that *P. aeruginosa* (Hill, 1978) as the predominant species in petroleum product which is in accordance with this research. This is expected because the genus is commonly found everywhere especially in hydrocarbon polluted area (Fought and Westlake, 1988). The total heterotrophic bacteria differ from those of the hydrocarbon utilizing bacteria when compared. This is due to the ability of the heterotrophic bacteria to withstand stress with time and have resided in the water phase where little nutrient is available. Though there were appropriate bacterial population in the samples, plastic degradation is near impossible if necessary nutrients were not available.

Conclusion

The results of the research have shown evidence of polystyrene plastic degradation which is in accordance with previous researches. Time series degradation processes by indigenous microorganisms from the soil have shown to be relatively efficient in the breaking down of plastics products as evidently indicated by the physicochemical analysis.

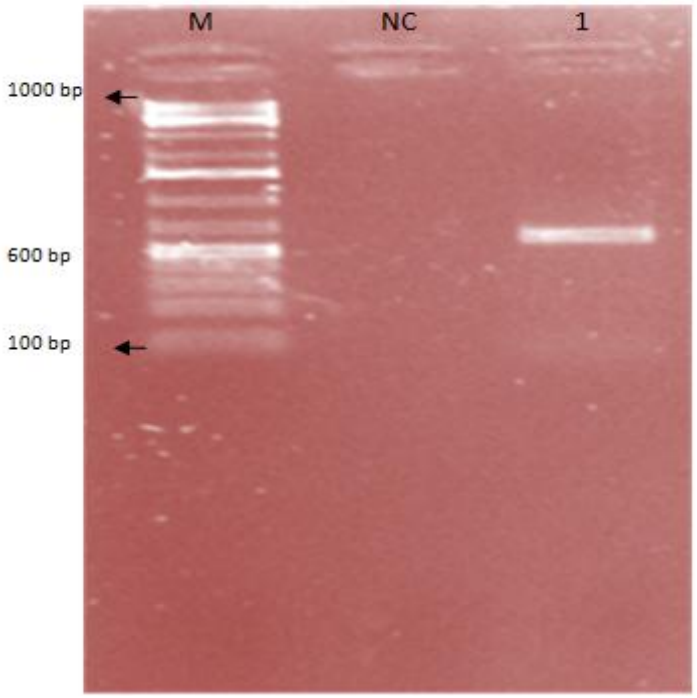

Plate 1. Polymerase chain reaction results for bacterial isolate analyzed with 1.5% agarose gel electrophoresis. M is 100 bp-1 kb DNA ladder (molecular marker). Lane 1 is positive for *Bacillus subtilis* with band at 600 bp. NC is a no DNA template control.

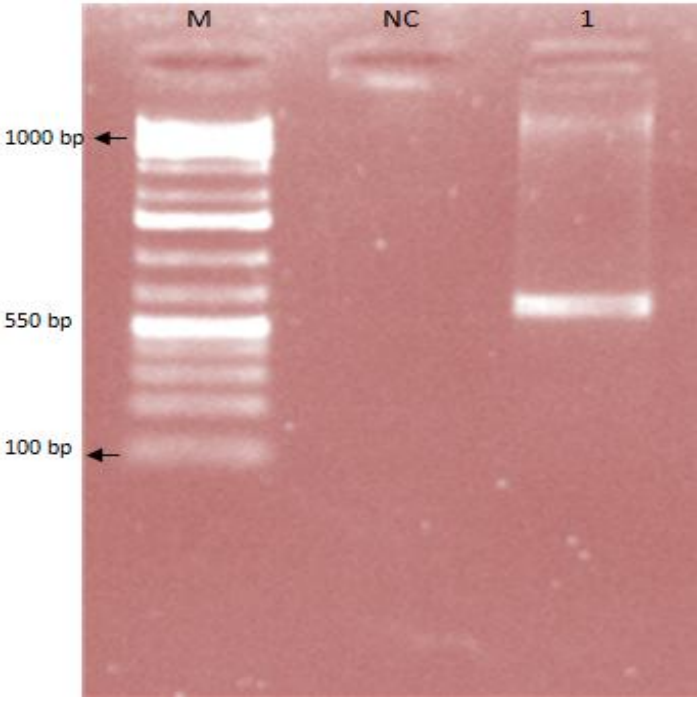

Plate 2. Polymerase chain reaction results for bacterial isolates analyzed with 1.5% agarose gel electrophoresis. M is 100bp-1kb DNA ladder (molecular marker). Lane 1 is positive for *Pseudomonas aeruginosa* with band at 550bp. NC is a no DNA template control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adamcova D, Vaverkova M (2014). Degradation of biodegradable/degradable plastics in municipal solid-waste landfill. Polyer Journal of Environmental Study 23:1071-1078.

Andrady AL, Neal MA (2009). Applications and societal benefits of plastics. Philosophical Transaction of the Royal Society of Biologist 364:1977-1984.

Atiq N, Safia A, Ali MI (2010). Isolation and identification of polystyrene biodegrading bacteria from soil. African Journal of Microbiological Resource 4:1537-1541.

Atuanya EI, Aborisade WT, Nwogu NA (2012). Impact of Plastic Enriched Composting on Soil Structure, Fertility and Growth of Maize Plants. European Journal of Applied Sciences 4(3):105-109.

Atuanya EI, Nwogu NA, Akpaje EO (2011). Biodegradation of Polyethylene Film by White-Rot Fungus *Pleurotus tuberrgium*. Nigerian Journal of Applied Science 29:19-25.

Atuanya EI, Udochukwu U, Daveomoregie AO, Inetianbor J (2016). Toxicological effects of plastic composted soil on nitrifying bacteria. British Microbiology Research Journal 13(4):1-7.

Ho BT, Timothy KR, Steven L (2018). An overview on biodegradation of polystyrene and modified polystyrene: the microbial approach. Critical Reviews in Biotechnology 38(2):308-320.

Baggi G, Boga MM, Catelani D (1983). Styrene catabolism by a strain of *Pseudomonas fluorescens*. Systematic and Applied Microbiology 4:141-147.

Brosius J, Dull TL, Sleeter DD, Noller HF (1981). Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli.* Journal of Molecular Biology 148:107-127.

Brulle F, Morgan AJ, Cocquerelle C, Vandelbulcke F (2010). Transcriptomic underpinning of toxicant-mediated physiological function alterations in three terrestrial invertebrate taxa: A review. Environmental Pollution 158(9):2793-2808.

Burkhard S, Bernd MR, Enrico M (2001). The Arabidopsis thaliana ABC transporter AtMRP5 controls root development and stomata movement. Environmental Microbiology Journal 20:1875-1887.

Coors A, Jones PD, Giesy JP, Ratte HT (2003). Removal of estrogenic activity from municipal waste landfill leachate assessed with a bioassay based on reporter gene expression. Environmental Science and Technology 37:3430-3434.

Ferrara G, Loffredo E, Senesi N (2006). Effects of bisphenol A on the microtubule arrays in root meristematic cells of *Pisum sativum*. Plant Letters 223:910-916.

Fought JM, Westlake DWS (1988). Degradation of Polycyclic Aromatic Hydrocarbon and Aromatic Heterocycles By *Pseudomonas* species. Canadian Journal of Microbiology 34:1135-1141.

Hill EC (1978). Microbial Degradation of Marine Lubricants, Its Detection and Control. Transactions of the Institute of Marine Engineers 90:197-216.

Himani B, Richa G, Archana T (2013). Communities of microbial enzymes associated with biodegradation of plastics. Journal of Polymers and the Environment 21:575-579.

Hwang JW, Choi CY, Park S (2008). Biodegradation of gaseous styrene by *Brevibacillus* sp. using a novel agitating biotrickling filter. Biotechnology Letters 30:1207-1212.

Jonsson S, Ejlertsson J, Ledin A, Mersiowsky I, Svensson BH (2003). Mono- and diesters from *o*-phthalic acid in leachates from different European landfills. Water Recourses 37:609-617.

Kaplan DL, Roy H, Jim S (1979). Biodegradation of Polystyrene, poly(metnyl methacrylate), and phenol formaldehyde. Applied Environmental Microbiology 38:551-553.

Mooney A, Ward PG, O'Connor KE (2006). Microbial degradation of styrene: biochemistry, molecular genetics, and perspective for biotechnical applications. Applied Microbiology and Biotechnology 72:1-10.

Mor R, Sivan A (2008). Biofilm formation and partial biodegradation of polystyrene by the actinomycete Rhodococcus ruber: Biodegradation of polystyrene. Biodegradation 19:851-858.

Motta O, Proto A, De Carlo F (2009). Utilization of chemically oxidized polystyrene as co-substrate by filamentous fungi. International Journal of Hygiene and Environmental Health 212:61-66.

Nishida H, Tokiwa Y (1994) Confirmation of poly(1,3-dioxolan-2-one)-degrading microorganisms in the environment. Chemical Letters 3:421-422.

Odokuma LO, Okpokwasili GC (1993). Role of Composition in the Degradability of Oil Spill Dispersants. Waste Management 12:39-43.

Oikawa E, Linn KT, Endo T (2003). Isolation and characterization of polystyrene degrading microorganisms for zero emission treatment of expanded polystyrene. Environmental Engineering Research 40:373-379.

Okpokwasili GC, Okorie BB (1991). Influence of Physicochemical Stress of Biodegradability of Car Engine Lubricating Oil. International Bioterrorism 27:255-264.

Opere BO, Ojo JO, Omonigbehin E, Bamidele M (2013). Antibiotic susceptibility and plasmid profile analysis of pathogenic bacteria isolated from environmental surfaces in public toilets. Transitional Journal of Science and Technology 3(2):22-30.

Sielicki M, Focht DD, Martin JP (1978). Microbial degradation of [C14C]polystyrene and 1,3-diphenylbutane. Canadian Journal of Microbiology 24:798-803.

Tischler D, Eulberg D, Lakner S (2009). Identification of a novel self-sufficient styrene monooxygenase from *Rhodococcus opacus* 1CP. Journal of Bacteriology 191:4996-5009.

Yang Y, Yang J, Wu WM (2015). Biodegradation and mineralization of polystyrene by plastic-eating mealworms (Part 2): Role of gut microorganisms. Environmental Science and Technology 49:12087-12093.

Zheng Y, Yanful EK, Bassi AS (2005). A review of plastic waste biodegradation. Critical Reviews in Biotechnology 25:243-250.

Related Journals: