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Plasmid- Mediated Antibiotic Resistance Associated with Health Care Workers (HCW) at the Federal University of Agriculture Abeokuta Clinic Institution-Based Cross-Sectional Study

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Abstract
Plasmid-mediated antibiotic resistance is a significant problem in treating infectious diseases. Health Care Workers (HCWs) are among the significant professional personnel involved in administering antibiotics. Most studies on antibiotic resistance among HCWs are questionnaire-based compared with *in vitro* studies with this group of people. This study aimed to characterize the antibiotic resistance pattern among health workers at the University. Clean catch midstream urine specimens were collected in sterile plastic vials from healthcare professionals. Samples were enriched in Selenite F broth and nutrient broth and incubated for 24 hours at 37^{o}c, an entire loop culture from turbid broths was inoculated onto appropriate agar plate. Isolated bacteria were screened for antimicrobial sensitivity using Kirby-Bauer disc diffusion method. Carbapenem[p\[resistant Enterobacteraceae (CRE) producers was detected by the modified hodges l,'',';.test (MHT). A plasmid curing (elimination) test was performed to determine the antibiotic resistant marker. Both descriptive methods of analyzing data were used in Statistical Analysis. More samples were recruited from the female gender (39), with the highest frequency in the age group of 21-30 and 41-50 (13 each). *K. pneumoniae has* the highest occurrence followed by *E.coli Pseudomonas aeruginosa* and *Enterobacter aerogenes*. Eleven isolates were resistant to imipenem but were producers of carbapenemase. Only 5 of the imipenem-resistant isolates showed susceptibility to the previously resisted drugs, while the remaining was plasmidic. Most Multidrug Resistance isolates from healthcare workers were Plasmidic in nature, suggesting that this resistance was horizontally transferred.

Keywords: Plasmidic; antibiotic resistance; Carbapenem-resistant *Enterobacteriaceae* (CRE); Health Care Workers (HCWs).

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Introduction

Bacterial plasmids are the custodian of genes responsible for resistance to antibiotics. Plasmid-mediated antibiotic resistance is a significant problem in treating infectious diseases (Letchumanan *et al*., 2015). The majority of genetic determinants that holds resistance to antibiotics are plasmid located. Extrachromosomal plasmids majorly mediate transmitted antibiotic resistance in bacteria and can be inherited by other bacteria in the surroundings by vertical or

horizontal gene transfer (Manjusha and Sarita, 2011). Horizontal gene transfer is essential in the rising and transferring resistance genes among species, including the transfer of resistance genes from coliform to community bacteria (Baquero *et al*., 2008). The specificity of plasmids in antibiotic resistance was initially reported from Japan when susceptible and multi-resistant (MDR) strains of microorganisms were cultured from the same person in a phase of a single outbreak of dysentery, hence, suggesting that the sensitive strains were becoming MDR, which was not a successive mutational advancement, instead by addition of the necessary genetic determinants in a one-step procedure. According to Watanabe and Fukasawa (1963), the route was a resultant effect of a transfer of plasmid initially referred to as resistance transfer factor (RTF) or R-factor, which hosts the resistance genes (Ramirez *et al*., 2014). Antimicrobial resistance (AMR) is a generalized health challenge that compromises the best management of infectious diseases (Chukwu *et al*., 2021). Health Care Workers (HCWs) are among the significant professional personnel involved in specifying, distributing, and administering antibiotics; hence, they are critical in managing infectious diseases (Barchitta *et al*., 2021). The role of healthcare professionals must be balanced in the effective use of antimicrobials (Simegn *et al*., 2022). Indeed, most of the epidemiological and clinical burden of infections caused by antibiotic-resistant microorganisms is associated with healthcare across the European Union (EU) (2017). However, AMR differs significantly in healthcare settings depending on the bacteria species, antibiotic class, and geographical region (Barchitta *et al*., 2021). In a clinical setting, where proper infection control steps are not in place, bacteria can spread from one patient to another through contact with surfaces (Saliba *et al*., 2021) or the unkempt hands of healthcare givers (Grabowski *et al*., 2017). Clinically relevant antimicrobial resistance is globally increasing in the environment, which is due to inappropriate use, the high load of infectious diseases, poor infection prevention, poor infection control, poor quality drugs, inadequate AMR knowledge, incorrect diagnosis, and absence of laboratory tests for drug susceptibility antimicrobials (Simegn *et al*., 2022). The World Health Organization (WHO) keeps advising HWs and consistently increasing awareness in healthcare settings as a top-notch strategy to reduce the rate of emergence and transmission of AMR (Chukwu *et al*., 2021). Most studies on antibiotic resistance among HW are questionnaire-based compared with *in vitro* studies with this group of people. Hence, this study aimed to characterize the antibiotic resistance pattern among health workers at the University.

Materials and Methods

Study Design, Setting, and Period

A cross-sectional research study was conducted among healthcare professionals at the Federal University of Agriculture Abeokuta Health Center, Nigeria. The land area of FUNAAB covers about 10,000 hectares of the North Eastern end of Abeokuta, the capital of Ogun State, Nigeria. The hospital serves thousands of people in the catchment area. It has about 100 health professionals at the time of the study period.

Study Population

Most health professionals working in diverse departments in the Health Center of the Federal University of Agriculture Abeokuta, Nigeria, were included in the study as the population source.

Inclusion and Exclusion Criteria

Health professionals who were on leave during the sample collection were also excluded.

Ethical consideration

The ethical committee of the Department of Microbiology at the Federal University of Agriculture Abeokuta, Nigeria, approved the study and sent an introductory letter to the school Health center about the study. Ethical approval was obtained from the Ogun state health ministry in Oke- Mosan, Abeokuta, with the ID number: HPRS/381/423.

Specimen collection

Clean catch midstream urine specimens were collected in sterile plastic vials and placed in an insulated box at 4°C before transporting to the Microbiology Department of the Federal University of Agriculture Abeokuta, Nigeria laboratory.

Isolation and identification of bacteria

Samples were enriched in Selenite F broth and nutrient broth. After 24 hrs incubation at 37oC, an entire loop culture from turbid broths was inoculated onto *Salmonella-Shigella* agar (Himedia, India), Hektoen enteric agar (Himedia, India) plates, MacConkey agar media, and EMB agar. After overnight incubation at 37oC, the pure cultures were further processed for morphological and biochemical characterization. Kirby-Bauer disc diffusion method was employed in determining the Antibiogram of all bacteria isolated from urine samples. Isolated bacteria were screened for antimicrobial sensitivity with the listed antibiotics: amikacin (AK) 30µg, amoxicillin (AMP) 25µg, ciprofloxacin (CIP) 30µg, cefotaxime (CTX) 30µg, doxycycline (DO) 30µg, D (DA) 2µg, enrofloxacin (ENR) 30µg, sulphamethoxazole/trimethoprim (SXT) 25µg, piperacillin/tazobactam (TZP) 110µg, and ofloxacin (OFX) 5µg. Briefly, lawns of bacteria were prepared for each isolate with standardized 0.5 McFarland overnight fresh bacterial suspension on Muller Hinton agar plates. All antibiotic discs were placed at equal distances. After 24 hrs incubation at 37oC, inhibition zones around antibiotics discs were measured and compared to Clinical Laboratory Standards Institute (CLSI, 2018).

Detection and characterization of carbapenem-resistant *Enterobacteriaceae* (CRE)

The Modified Hodges Test (MHT) was employed for detecting CRE. Briefly, A 0.5 McFarland dilution of E. coli ATCC 25922 in 5ml of sterile water was prepared and streaked as a lawn on a Mueller Hinton agar plate. A meropenem susceptibility disc was placed in the center of the plate. The test organism is streaked to form a straight line from one edge of the disc to the edge of the plate. The test organism plate was incubated for 24 hours at 37°C in ambient air for 16 to 24 hours. After 24 hours, MHT positive test shows a clover leaf-like indentation of the Escherichia coli 25922 growing along the test.

Plasmid healing Assay

A plasmid curing (elimination) test was performed to determine the location (plasmid-borne or chromosomal) of the AR marker (s) using sublethal concentrations of ethidium bromide as described by Bouanchaud *et al*. **(**1968**)** with slight modifications. Briefly, overnight culture of selected MDR bacterial isolates colonies showing resistance to a minimum of three antibiotics was inoculated into 5 mL of an enrichment broth (nutrient broth) containing different concentrations of curing agent (ethidium bromide) (50, 75, and 125 µg/mL) and incubated at 370c for 24 h . Serial dilutions were made, seeding the cultures onto prepared Müller Hilton agar plates and incubating at 37 °C for 3 hours before placing the discs at the center of the plates. The plates were kept incubated at 36°C for 2 days, and the inhibition zones were read. The absence of an inhibition zone on agar Mueller-Hinton indicates plasmid resistance (plasmid cured). In contrast, an inhibition zone to the previously resistant antibiotics signals chromosomic resistance (plasmid not healed).

Statistical Analysis

Both descriptive methods of analyzing data were used. The descriptive analysis includes tables, charts, and simple frequency distribution.

Results

A total of 60 urine samples were collected during the study. More samples were recruited from the female gender (39), with the highest frequency in the age group of 21-30 and 41-50 (13 each) (Table 1). The highest number of samples were obtained in the nursing unit (23), followed by the cleaner (6), social worker and record (5), laboratory and pharmacy (4), doctor and driver (3 each), security (2) and accountant, health officers, NHIS and X-ray with 1 each. (Table 2). *K.*

pneumoniae has the highest occurrence among females, with 11 (32.4%) isolates, while *P. aeruginosa has* the lowest occurrence, with 5 isolates (14.7%). Among the males, both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* has the highest occurrence with 4 isolates each (36.4%), while *Escherichia coli* has the lowest occurrence with 1 (9.1%) isolate (Table 3). The age group of 21-30 has the highest frequency for *Escherichia coli* with 4 isolates. Age group 31-40 had the highest frequency for *Pseudomonas aeruginosa* with 3 isolates. Groups 31-40 had the highest frequency for *Enterobacter aerogenes* with 4 isolates. Group 41-50 has the highest frequency for *Klebsiella pneumoniae* with 8 isolates (Figure 1).

Enterobacter aerogenes showed more resistance to most antibiotics, but *Klebsiella pneumoniae* was more susceptible to antibiotics (Table 4). Six isolates of *Enterobacter* aerogenes were resistant to carbapenem drug (Imipenem), followed by *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Table 5). Modified Hodges test reveals that none of the CRE isolates were producers of carbapenemase (Table 6). Out of 11 CRE isolates subjected to plasmid curing, only 5 showed susceptibility to the previously resisted drugs, while the remaining was plasmidic.

Table 1: Distribution of Urine Samples among Age group

Sex	21-30	31-40	41-50	51-60	61-70	Total
Male	1(4.8%)	10(47.6%)	6(28.6%)	3(14.3%)	1(4.8%)	21(100%)
Female	12(30.8%)	10(25.6%)	7(17.9%)	7(17.9%)	3(7.7%)	39(100%)
Total	13(21.7%)	20(33.3%)	13(21.7%)	10(16.7%)	4(6.71%)	60(100%)

Table 2: Distribution of Gender Across units in the Health Centre

	Male	Female	Total
Accountant	0(0.0%)	1(100.0%)	1(100.0%)
Cleaner	1(16.7%)	5(83.3%)	6(100.0%)
Doctor	3(100.0%)	0(0.0%)	3(100.0%)
Driver	3(100.0%)	0(0.0%)	3(100.0%)
Health officer	0(0.0%)	1(100.0%)	1(100.0%)
Laboratory	1(25.0%)	3(75.0%)	4(100.0%)
NHIS	1(100.0%)	0(0.0%)	1(100.0%)
Nursing	1(4.3%)	22(95.7%)	23(100.0%)
Pharmacy	2(50.0%)	2(50.0%)	4(100.0%)
Record	2(40.0%)	3(60.0%)	5(100.0%)
Secretary	0(0.0%)	1(100.0%)	1(100.0%)
Security	2(100.0%)	0(0.0%)	2(100.0%)
Social worker	4(80.0%)	1(20.0%)	5(100.0%)
x-ray	1(100.0%)	0(0.0%)	1(100.0%)
Total	21(35.0%)	39(65.0%)	60(100.0%)

Table 3: Distribution of Bacteria among Gender.

	E. coli	*P. aeruginosa*	*E. aerogenes*	*K. pneumoniae*	Total
Male	1(9.1%)	4(36.4%)	2(18.2%)	4(36.4%)	11(100%)
Female	10(29.4%)	5(14.7%)	8(23.5%)	11(32.4%)	34(100%)
Total	11(24.4%)	9(20.0%)	10(22.2%)	15(33.3%)	45(100%)

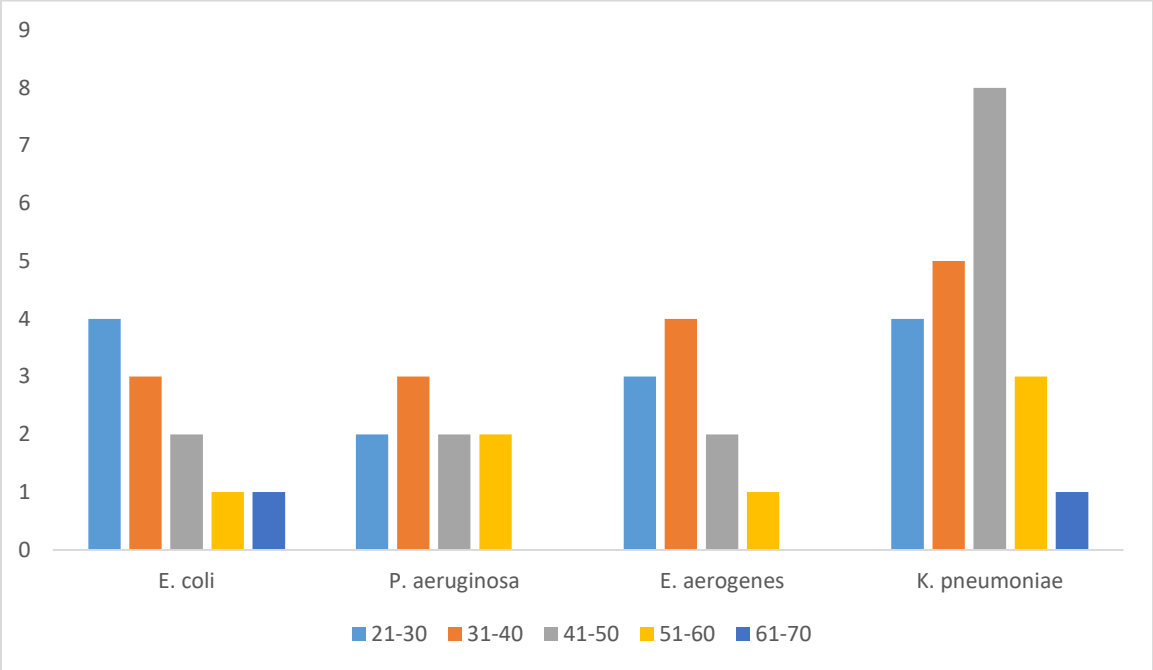

Figure 1: Distribution of Bacteria obtained among Age groups.

Key:
E. coli: *Escherichia coli* P. aeruginosa: *Pseudomonas aeruginosa*
E. aerogenes: *Enterobacter aerogenes* K. pneumoniae: *Klebsiella pneumoniae*

Table 4: Antibiotics Susceptibility Pattern on Antibiotics discs.

	Total	**OFX**		**PEF**		**CN**		**AU**		**AM**		**CPX**		**SP**		**CH**		**SXT**		**S**	
		R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Ec	11	3	0	1	2	1	2	3	0	3	0	1	2	1	2	3	0	3	0	3	0
Pa	9	2	1	1	2	1	2	2	1	2	1	0	3	1	2	2	1	1	2	0	3
Ea	10	4	3	4	3	4	3	6	1	4	3	5	2	3	4	3	4	3	4	3	4
Kp	15	3	0	1	2	2	1	3	0	3	0	2	1	2	1	2	0	3	0	3	0
Total	45	12	4	7	9	8	8	14	2	12	4	8	8	7	9	1	5	10	6	9	7

Key:

Ec: *Escherichia coli* Pa: *Pseudomonas aeruginosa* Ea: *Enterobacter aerogenes*
Kp: *Klebsiella pneumonia* Tarivid (OFX): 10ug Pefloxacin (PEF): 30ug
Gentamycin (CN): 10ug Augmentin (AU): 30ug Amoxicillin (AM): 30ug
Ciprofloxacin (CPX): 10ug Sparfloxacin (SP): 10ug Chloramphenicol (CH):30ug
Sceptrin (SXT):30ug Streptomycin (S): 30ug

Discussion

Antibiotic resistance is a global issue that all and sundry must rise against it. Healthcare givers harboring antimicrobial-resistant organisms are the potent and fast routes of transmitting this menace. Most studies on antibiotic resistance among HW are restricted to questionnaires. This study explores the *in vitro* pattern of the bacterial isolates from this group of people. More isolates were from the female gender and the age group of 21-30. This age group often represents new staff in the profession, which suggests that they may not employ the necessary precautions needed

while discharging their duties. This correlates with the result of another study (Simegn *et al*., 2022), which studied the Knowledge of Antimicrobial Resistance and Factors among Health Professionals at the University of Gondar Specialized Hospital. The CRE isolated in this study were not carbapenemase producers indicating that the transmission of resistant genes was not via a person-to-person discharge of the gastrointestinal host of the resistant isolates. This study agrees with the study of Bitterman *et al*. (2016), who had a prospective study on the rate of colonization of healthcare workers by carbapenem-resistant Enterobacteriaceae in an endemic hospital. The most predominant bacteria in this study was *K. pneumonia*. More bacteria isolates were obtained from the female than from males. *Klebsiella pneumoniae* was the most isolated organism from the female. This agrees with the study of Suetens *et al.* (2018), which shows that the prevalence of *Klebsiella pneumoniae* in CRE infections in hospitals was higher in females than in males. This contradicts some past research works, such as Uma *et al.* (2009), who isolated 40% E.coli in their study on antibiotic sensitivity and plasmid profiles of *E.coli* Isolated from pediatric diarrhea. Also, Uddin *et al*. (2021) isolated *Salmonella* spp. (43%), and *Shigella* spp. (43%) in the study on Bacterial gastroenteritis in children. These differences might be attributed to the type and source of samples taken. The antibiogram results of this study showed that some of each bacterial isolates were susceptible to Pefloxacin (PEF) 30ug, Gentamycin (CN) 10ug, Ciprofloxacin (CPX) 10ug, and Sparfloxacin (SP) 10ug. Most of the MDR isolates in this study were plasmid-mediated. This correlates with the research of Tenea *et al*. (2023), whose findings reveal more plasmid-mediated bacterial isolates. Therefore, it is imperative to consider this group (Health Workers) of the public as a subject to be given priority in the effort to reduce antimicrobial resistance.

Conclusion

Most MDR isolates from healthcare workers were Plasmidic, suggesting that this resistance was horizontally transferred. There will be need for intentional effort of the health workers to avert healthcare associated infections which might be a vehicle of resistance development within the health-care settings and invariably to the community they tends to care for.

Declaration of Conflict of Interest

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The authors have no relevant financial or nonfinancial interests to disclose.

Authors' contributions

All authors listed on the manuscript contributed significantly to the experimental design, its implementation, or analysis and interpretation of the data.

Credit author statement

OJO A.E: Conceptualization, Methodology, Original draft preparation; **OJO O.A.**: Supervision, Writing- Reviewing and Editing**; Adebajo S.O. Investigation Software, Validation.** ***Ajibola A.T.*** Supervision, Methodology; **Balogun B.A. Data curation, laboratory analysis Oloyede A.R. Software** Validation and Reviewing and Editing: **Ojo D.A.:** Writing-Supervision, Reviewing and Editing

Declarations

Ethics approval and consent to participate: Ethical approval (HPRS/381/423.) was obtained for this study from the ethical committee of the hospital where the research was conducted.

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