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Multiple antibiotic resistant index and detection of qnrS and qnrB genes in bacterial consortium of urine samples from clinical settings

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ABSTRACT: The multiple antibiotic resistant (MAR) index and detection of resistant genes in the bacterial consortium of urine samples collected from University of Medical Sciences Teaching Hospital, Akure (UNIMEDTH) was evaluated with all microbiological and biotechnological techniques employed utilizing specified standards in this study. *Escherichia coli* had the highest bacterial count (311.50 ± 0.707 CFU/ml) while *Staphylococcus saprophyticus* had the least (13.00 ± 2.828 CFU/ml). *Enterococcus faecalis*, and *Pseudomonas aeruginosa* isolate showed marked resistance against four classes of antibiotics tested. The MAR index of bacterial isolates ranged from 0.5 to 1.0. Fluoroquinolone-resistant *P. aeruginosa* identified to be *P. aeruginosa* via 16S rDNA analysis sequence analysis of 417 base pairs with strain mcbay1 deposited in GenBank with accession number MT423976 was positive for qnrS resistant gene. *E. faecalis* identified by 16S rRNA sequence analysis of 264 bp of the strain mcbay 2 deposited in GenBank with accession number MT423977 was also positive for qnrB resistant gene. The presence of resistant genes in ciprofloxacin-resistant *P. aeruginosa* and quinolone-resistant *E. faecalis* in urine samples further emphasized the need for the regulation of over-the-counter prescription and antibiotic susceptibility survey of anti-pseudomonal and anti-enterococcal quinolones in hospital settings.

Keywords: Antibiotics; Anti-pseudomonal; Resistance; Resistant genes; Urine, Quinolones.

1. INTRODUCTION

There is a disturbing rise of chemotherapeutic resistance in bacteria that provoke nosocomial infections as stated by Shaikh et al. [1]. Multidrug resistant bacteria can be amassed in numerous environmental alcoves because of the rife use of commercially-available antibiotics in the hospices, agriculture and in livestock. Multiple antibiotic resistances (MARs) in bacteria may be generally related with the occurrence of plasmids as opined by Jernberg et al. [2]. Resistant infections are becoming more complicated or even impracticable to treat with contemporary drugs, consequently infections causing higher morbidity, eliciting huge costs on the society as demonstrated by Finley et al. [3]. This growing resistance involves several frequent human pathogens, including *Escherichia coli, Staphylococcus aureus,* of *Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter*, *Enterococcus* species and other multidrug bacterial consortia as shown by Finley et al. [3].

Exposure of ecological bacteria to antimicrobials and to great numbers of resistant bacteria may hasten the fruition of resistance, increase the profusion and circulation of resistant genes within the reservoir that is decisive to the development of clinical resistance, and increase substitute of antibiotic resistance genes between bacteria as stated by Poirel et al. [4]. The genesis for a number of the main challenging resistance genes is marine organisms such as *Shewanella* species, which carries gene-programming quinolone from Enterobacteriaceae infections, particularly *E. coli* and *Salmonella* Typhi resistance genes (qnr) on its chromosome as affirmed by Gillings and Stokes [5]. In humans, plasmid-mediated qnr genes are more commonly recognized in species. It is apt progressively more recognized that not only antibiotic resistance genes (ARGs) identified in clinical pathogens are of significance, but relatively, all infectious and ecological bacteria, mobile genetic elements (MGEs) and bacteriophages, form a repertoire of ARGs from which pathogenic bacteria can obtain resistance through horizontal gene transfer (HGT) as stated by Von Wintersdorff [6].

Quinolones are a set of antibiotics that are able to impede with DNA (deoxyribose nucleic acid) duplication and transcription in bacteria as affirmed by Etebu and Arikekpar [7]. Their makeup generally consists of two rings but recent generations of quinolones possess an added ring structure which enables them to expand their range of antimicrobial activity to some bacteria, predominantly anaerobic bacteria that were up till now resistant to quinolones. Horizontal gene transfer (HGT) can be influenced by human-related conduct whereby gene transfers amid different organisms made probable by the introduction of selective pressures in the line of antibiotic abuse/misuse that allow the continuum of transferred genes as opined by Gillings [8]. *Pseudomonas aeruginosa* is one of the principal causes of nosocomial urinary tract infections (UTIs). It can overrun the circulatory system from the urinary tract, and this has been demonstrated to be the bane of nearly 40 % of *Pseudomonas* bacteremia. Urinary tract infections caused by *P. aeruginosa* are usually hospice-associated as stated by Bekele et al. [9].

There are diverse pools obtainable for antibiotic-resistant enterococci. One of the main pools is hospice settings where antimicrobials are extensively used. To prohibit potential enterococcal bugs, multifarious and diverse classes of chemotherapeutic agents are given consequently leading to selective stress for bacteria using inherent or environmentally-implicated antibiotic resistant means. Excess usage of antimicrobials also plays an significant part in the assessment of MDR enterococci as opined by Miller et al. [10] for that reason, heightened levels of chemotherapeutic resistance in enterococci species predominantly *E. faecalis* and *E. faecium* to several dissimilar classes of antibiotic must not be disregarded as affirmed by Weng et al. [11].

Infections caused by *Pseudomonas aeruginosa* are mounting both in hospital and in the society and it has been detailed as one of the chief causes of nosocomial pathogen, predominantly amongst immune-compromised patients as earlier stated by Bekele et al. [9]. Therefore, this study was carried out to determine the multiple antibiotic resistant (MAR) index and detect the presence of resistant genes including qnrS, qnrB genes and other genes in bacterial consortium of urine samples from University of Medical Sciences Teaching hospital, Akure, Nigeria.

2. MATERIALS AND TECHNIQUES

2.1. Study area description

University of Medical Sciences Teaching Hospital Complex, Akure (which was formerly called State Specialist Hospital) with coordinates 7.2421º N, 5.1957º E is located at hospital road, Akure in Akure South Local Government Area of Ondo State (Figure 1). It is a state-owned teaching hospital that has being recently merged with other state-owned health centres in the state. An estimated number of more than 3,000 patients attend this hospital on a weekly basis.

2.2. Collection of urine samples from UNIMEDTH

206 urine samples were collected in sterile bottles from Microbiology department of Medical laboratory unit, University of Medical Sciences Teaching Hospital, Akure, Nigeria. They were transported at a temperature of 4-8 ºC in a coolant pack to the Microbiology Department laboratory of the Federal University of Technology, Akure (FUTA) and analyzed within 1 hr of collection as demonstrated by Geoffrey et al. [12].

2.3. Isolation of bacteria in urine samples from UNIMEDTH

The urine samples were immediately transferred aseptically using sterile and flamed wire loop by streaking unto MacConkey agar (Hi-Media, India), Blood agar (Hi-Media, India), Mannitol Salt Agar (MSA) (Hi-Media, India) and Cysteine Lactose Electrolyte Deficient Agar (CLED) (Hi-Media, India), which were considered as selective and differential medium for the isolation and identification of Enterobacteriaceae and Staphylococci species. Petri plates were incubated at 37 ºC for 24 hr, then a single pure isolated colony was transferred to nutrient agar medium for preservation and further authentication and identification was carried out as juxtaposed by Atlas [13].

2.4. Biochemical tests for the identification of bacterial isolates

All the bacterial isolates were initially identified using standard biochemical techniques as described by Hemraj et al. [14]. First, all isolates were sub-cultured on nutrient agar plates and incubated at 35ºC for 24 h. In all the biochemical tests, un-inoculated tubes were used as controls. Gram stain, catalase, coagulase, motility, hydrogen sulphide production, urease, indole oxidase and citrate tests were employed as glucose, sucrose, lactose and mannitol were sugar tests conducted.

2.5. Antibiotic sensitivity test of the urine bacterial isolates

Antibiotic sensitivity tests were performed on the bacterial isolates using standard agar diffusion protocols as described by Clinical Laboratory Standard Institute (CLSI, 2017), [15] with commercially available antibiotics. All sensitivity tests were carried out using overnight cultures. A 1ml suspension of each bacteria isolates, equivalent to McFarland standards was aseptically seeded into Mueller Hinton agar (MHA) (Hi-Media, India) plates respectively. This was allowed to stand for one hour to solidify. The antibiotic disc profile containing Cefuroxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Augmentin (30 µg), Gentamycin (10 µg), Ofloxacin (5 µg), Ciprofloxacin (5 µg), and Nitrofurantoin (300 µg) (Oxoid, UK) were aseptically placed on the surface of the molten (MHA) (Hi-Media, India) and allowed for 30 mins to pre-diffuse. The set up was done in triplicate for each isolate, with a control plate containing no antibiotic disc. These were incubated for 18-24 hr at 37ºC, after which the diameter of zone of inhibition was measured using a vernier caliper (Delson Pascal Nigeria Ltd) and the results were interpreted using standard interpretative charts as recommended by CLSI [15]. Multidrug resistance was indicated by resistance to a minimum of three different antibiotics.

2.6. Determination of multiple antibiotics resistance (MAR) index of bacterial isolates

The MAR index was computed as the fraction of the quantity of antimicrobials which an isolate is resistant to (a), the sum quantity of antimicrobials to which the isolates were used against (b). MAR = a/b.

2.7. Antibiotic resistance gene assay

2.7.1. DNA preparation

Polymerase chain reaction with definite primers were used to categorize gyrA, qnrA, qnrS and qnrS2 genes of the ciprofloxacin-resistant *Pseudomonas aeruginosa* isolates shown in Table 1. They were purchased from Inqaba Biotec West Africa Ltd, located at Bioscience Unit, International Institute for Tropical Agriculture, Ibadan, Nigeria (IITA). DNA model was prepared as described by Olsvik and Strockbin [16]. 25 µl of PCR extension assortment containing 12.5 µl of deionized sterile water ((Thermo Fischer Scientific, UK) was utilized with Green Go Taq master mix (Promega, USA) [17]. PCR-sequencing was conducted DNA Sanger sequencing and data was analyzed by ABI Sequencing Analysis software (version 5.2).

Table 1. The primer sequences used for the detection of qnrA and qnrS genes in ciprofloxacin-resistant *P. aeruginosa* isolates.

Primer type	Primer sequence	Base pairs
gyrA	3-CCAGATGTTCGTGACGGTT-5	258
	3-ATTGCTGCTGCGCCATCTCC-5	
qnrS	5-ACGACATTCGTCAACTGCAA-3	417
	5-TAAATTGGCACCCTGTAGGC-3	

Table 2. Primer sequence used for the extension of quinolone-resistant genes in *E. faecalis* clinical isolates.

Target genes	Primer sequence '3-5'	Base pairs
qnrA	AGAGGATTTCTCACGCCAGG	580
	TGCCAGGCACAGATCTTGAC	
qnrB	GGMATHGAAATTCGCCACTG	264
	TTTGCYGYYCGCCAGTCGAA	
qnrS	GCAAGTTCATTGAACAGGGT	428
	TCTAAACCGTCGAGTTCGGCG	

2.7.2. Amplification of qnrA, qnrB and qnrS genes by PCR

Deoxyribose nucleic acid (DNA) of quinolone-resistant species *Enterococcus faecalis* extraction was done by boiling technique according to the strategy of Oyamada et al. [18] where Reactions were repeated for 30 cycles for 15 sec. at 94 ºC for denaturation, 30 sec. at 55 ºC for annealing, and 1 min. at 72 ºC for polymerization. PCR-sequencing was conducted by the cycle sequencing technique using an ABI Prism big dye terminator version 3.1 cycle sequencing kit, and then placed on a Genetic analyser 3100 (Applied Biosystems).

PCR extension of qnr genes (qnrA, qnrB, qnrS) purchased from Inqaba Biotec West Africa Ltd, located at Bioscience Unit, International Institute for Tropical Agriculture, Ibadan, Nigeria (IITA) were executed with the detailed primers as employed by Kim et al. [19] listed in Table 3. PCR products were investigated by electrophoresis (Cleaver Scientific, UK) in a 1.5% agarose gel (SYBR safety, Thermo Fischer Scientific UK) at a voltage of 100 Volts for duration of 30 min. Sequence breakdown and comparisons were carried out using programs available at the National centre for biotechnological information (NCBI) server.

2.8. Data analysis

All experiments were performed in triplicates and data derived from the study were subjected to 2-way analysis of variance (Anova) and level of significance was documented at $p \leq 0.05$. Separation of means was performed using Duncan's new multiple range test (DNMRT) at 95% confidence level for antibiotic sensitivity testing with the aid of SPSS (version '22). Urine colony count means were separated using mean ± standard deviation.

3. RESULTS

Bacteria were isolated from clinical urine samples including: *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis, Staphylococcus aureus* and *S. saprophyticus* as shown in Table 3. Gram negative bacilli accounts for 4 of the bacterial isolates while 3 were Gram positive cocci.

Table 3. Effect of IVM and/or AA on body weight and weight gain for the acclimation and experimental periods.

Probable organism	Catalase	Coagulase	Motility	H_2S	Urease	Indole	Oxidase	Glucose	Sucrose	Lactose	Mannitol	Citrate	Gran reaction
Escherichia coli	+	-	+	+	-	+	-	AG	A	+	+	-	-ve rods
Staphylococcus aureus	+	-	-	-	-	-	-	+	-	+	+	-	+ve cocci
Proteus vulgaris	+	-	+	+	+	-	-	+	+	-	-	+	-ve rods
Klebsiella pneumoniae	-	-	-	-	-	-	-	AG	+	+	+	+	-ve rods
Enterococcus faecalis	-	-	-	-	-	-	-	+	-	+	+	-	+ve cocci
Pseudomonas aeruginosa	+	-	+	-	-	-	+	-	+	-	+	+	-ve rods
Staphylococcus saprophyticus	+	-	-	-	-	-	-	+	-	+	+	-	+ve cocci

Key: + = Positive, - = Negative, A = Acid production, AG = Gas production.

3.1. Occurrence of urine bacterial consortium (CFU/ml)

The multiple antibiotic resistant (MAR) index and detection of qnrS and qnrB genes in bacterial consortium of urine samples from clinical settings in Akure were ascertained. *Escherichia coli* had the highest bacterial count of 311.50 ± 0.707 CFU/ml while *Staphylococcus saprophyticus* had the least (13.00 ± 2.828 CFU/ml) as shown in Table 4.

Table 4. Occurrence of bacteria in urine samples collected from UNIMEDTH (n = 206).

Organisms	CFU/ml
Escherichia coli	311.50 ± 0.707
Pseudomonas aeruginosa	93.00 ± 2.828
Klebsiella pneumoniae	74.50 ± 2.121
Proteus vulgaris	69.00 ± 1.414
Enterococcus faecalis	47.50 ± 0.707
Staphylococcus aureus	38.00 ± 1.414
Staphylococcus saprophyticus	13.00 ± 2.828

Values are means +/- standard deviation; Key: Colony Forming Units per millilitre (CFU/ml).

3.2. Antibiotic sensitivity pattern of urine bacterial consortium

Staphylococcus saprophyticus was sensitive to ofloxacin (5 µg) at 24.21 ± 3.06 mm and resistant to ceftriaxone (30 µg) at 7.33 ± 2.33 mm. *Staphylococcus aureus* was sensitive to ciprofloxacin (5 µg) at 22.10 ± 2.00 mm and resistant to ceftriaxone at 6.11±0.22 mm. *E. coli*, *P. aeruginosa* and *K. pneumoniae* were resistant to all tested antibiotics as presented in Table 5.

Table 5. Antibiotic sensitivity pattern of urine bacterial isolates.

Antibiotics	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*P. vulgaris*	*E. faecalis*	*S. saprophyticus*	*S. aureus*
NIT	16.00±0.0 ^{bc}	6.41±1.00^{ab}	7.33±0.67^{b}	18.00±2.04^{cde}	20.67±2.18^{ef}	12.67±2.67^{bcd}	20.00±1.15^{bc}
CPR	6.14±0.10^{ab}	6.23±0.20^{b}	6.22±0.65^{ab}	18.67±2.60^{bc}	14.67±4.67^{bc}	16.02±1.88^{bc}	22.10±2.00^{cd}
CAZ	6.20±0.22^{a}	6.10±1.23^{bc}	6.10±1.01^{bc}	15.38±2.40^{ab}	6.24±0.11^{ab}	17.33±1.33^{ab}	6.12±0.33^{a}
CRX	6.11±0.44^{b}	6.17±2.22^{ab}	6.33±1.67^{ab}	6.16±0.08^{bc}	6.21±2.00^{bc}	7.33±2.33^{ab}	9.33±1.67^{b}
GEN	6.22±1.19^{ab}	6.16±1.11^{ab}	6.19±0.56^{bc}	14.67±1.71^{a}	18.67±2.76^{ab}	16.00±1.00 ^{cd}	6.11±0.02^{a}
CXM	6.31±1.61^{bc}	6.09±0.21^{b}	6.00±1.50^{a}	6.04±2.04^{a}	6.23±0.22^{ab}	6.01±1.00^{a}	21.33±1.67cd
OFL	6.08±2.18^{ab}	6.13±1.14^{bc}	6.31±1.44^{bc}	18.22±3.16^{bc}	20.67±3.53^{cd}	24.21±3.06^{bcd}	15.33±1.33^{bc}
AUG	6.19±2.11^{a}	6.29±2.22^{ab}	6.12±0.09^{ab}	6.10±0.08^{a}	11.33±3.52^{bc}	6.03±0.21^{a}	6.16±1.04^{ab}

Figures carrying identical alphabets in the matching column are not extensively dissimilar, P≤ 0.05. Keys: NIT – Nitrofurantoin; CPR – Ciprofloxacin; CAZ – Ceftazidime, CXM – Cefuroxime; AUG – Augmentin; CRX – Ceftriaxone; OFL – Ofloxacin, GEN – Gentamycin.

3.3. Antibiotic susceptibility profile of urine bacterial isolates

Escherichia coli, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was found to be resistant to Nitrofurantoin, Ciprofloxacin, Ceftazidime, Cefuroxime, Augmentin, Ceftriaxone, Ofloxacin, and Gentamycin. *Proteus vulgaris*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* showed relative susceptibility and resistance against the tested antibacterials as represented in Table 6.

Table. 6. Antibiotic sensitivity pattern (according to CLSI standard).

Antibiotics	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*P. vulgaris*	*E. faecalis*	*S. saprophyticus*	*S. aureus*
S≥17; R≤14 NIT	R	R	R	S	S	R	S
S≥21; R≤15 CPR	R	R	R	I	R	I	S
S≥21; R≤17 CAZ	R	R	R	R	R	S	R
S≥23; R≤19 CRX	R	R	R	R	R	R	R
S≥15; R≤12 GEN	R	R	R	I	S	S	R
S≥18; R≤14 CXM	R	R	R	R	R	R	S
S≥16; R≤12 OFL	R	R	R	S	S	S	I
S≥15; R≤12 AUG	R	R	R	R	R	R	R

S – Susceptible; R – Resistant; I – Intermediate.

3.4. Multiple antibiotic resistant index (MAR) of urine bacterial consortia

Multiple antibiotic resistance (MAR) index of bacterial isolates from urine samples ranged from 0.5–1.0 as shown in Table 7.

Table 7. MAR index of multifarious antibacterial resistant urine bacterial isolates.

Bacterial codes	Resistant (a)	Tested (b)	MAR index (a/b)
UI1	8	8	1.0
UI2	5	8	0.63
UI3	5	8	0.63
UI4	5	8	0.63
UI5	8	10	0.8
UI6	10	10	1.0
UI7	7	10	0.7
UI8	7	10	0.7
UI9	10	10	1.0
UI10	6	10	0.6
UI11	5	10	0.5
UI12	6	10	0.6

Keys: a = the fraction of the quantity of antimicrobials which an isolate is resistant to; b = the sum quantity of antimicrobials to which the isolates were used against; UI = Urine isolate.

3.5. Biochemical and molecular depiction of bacterial consortia from urine specimens

Figure 1 shows the gel electrophoresis image of DNA fragments of bacterial isolates with 1.1 kilo base pairs. Isolate codes including; Ec, Kp and Pa were presumptively (biochemical) identified as *Enterococcus faecalis* and *Pseudomonas aeruginosa* respectively. *E. faecalis* was molecularly confirmed as *E. faecalis* strain Mcbay 2 with GeneBank accession number MT423977, while *P. aeruginosa* was molecularly confirmed as *P. aeruginosa* strain Mcbay 1 with GeneBank accession number MT423976 as shown in Table 8.

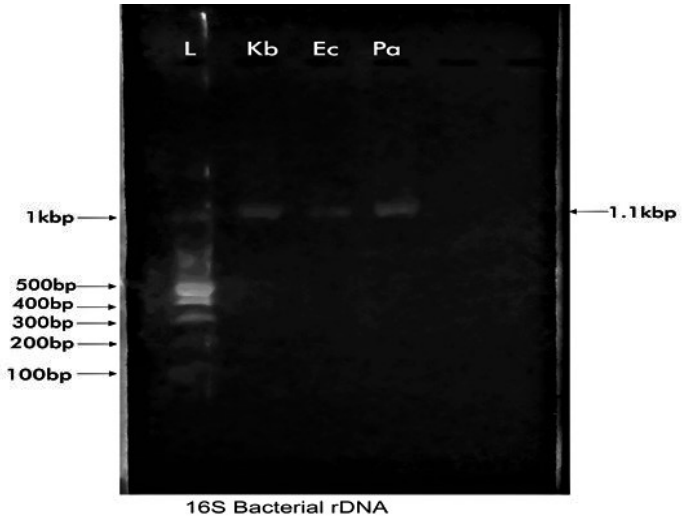

Figure 1. 1.1 kilo base pairs deoxyribose nucleic acid (DNA) amplicon bands of Kb, Ec and Pa. Key: L = Molecular weight marker.

Table 8. Molecular identification of urine bacterial isolates.

Isolate codes	Biochemical identity	Molecular identity	% similarity	Strain no.	Accession number
Ec	*Enterococcus faecalis*	*Enterococcus faecalis*	99.31%	Mcbay2	MT423977
Pa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	97.43%	Mcbay1	MT423976

3.6. Antibiotic resistant gene(s) depiction of selected bacterial consortia from urine samples

Ciprofloxacin-resistant *P. aeruginosa* strain Mcbay1 with GenBank accession number MT423976 tested for the presence of gyrA, qnrB, qnrS2 and qnrS genes by PCR showed QnrB was positive at 417 kilobase pairs (kbp) and *E. faecalis* strain Mcbay 2 with Gen Bank accession number MT423977 had qnrS gene positive at 264 kilobase pairs (kbp) as shown in Figure 2.

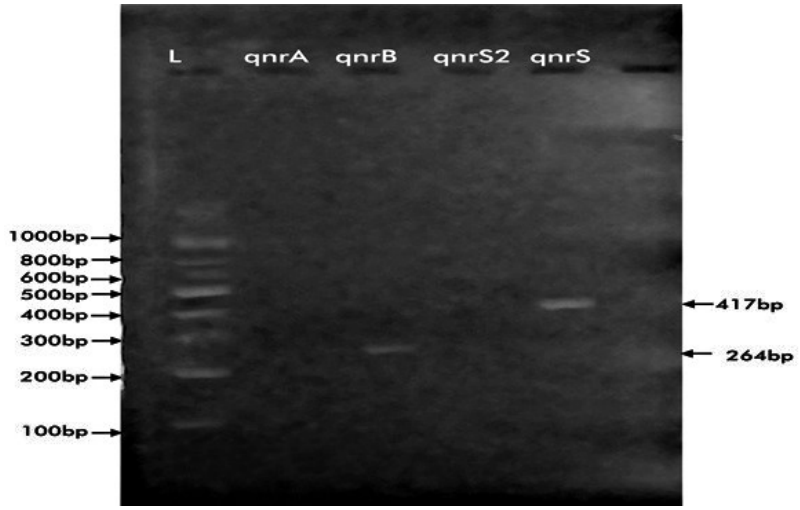

Figure 2. 1.2% agarose gel plate of positive qnrB and qnrS2 resistant genes amplified doubly with 264 base pairs and 417 base pairs respectively in molecularly-identified *Pseudomonas aeruginosa* MT423976 and *Enterococcus faecalis* MT423977, L=DNA marker.

4. DISCUSSION

The multiple antibiotic resistant (MAR) index of urine bacterial isolates from a clinical setting and the detection of the presence of antibiotic resistant genes in the bacterial conglomerate of urine samples from University of Medical Sciences Teaching hospital, Akure, Nigeria was ascertained. Bacterial isolates known to belong to the Enterobacteriaceae family were high among the two hundred and six (206) samples collected; this was consistent with the work of Chroma and Kolar [20]. The result of the biochemistry of bacterial load of urine specimens in this study correlates with the work of Huang et al. [21] as they enumerated *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis*, and *Enterococcus faecalis* from urine subjects. *Staphylococcus* species isolated from this study also correlates with the observations of Amin et al. [22] as they opined that coagulase negative Staphylococci are a common cause of urinary tract infection and *S. saprophyticus* tends to cause infection in young women of a sexually-active age. The commonest pathogenic organism isolated from urine samples is *Escherichia coli* followed by *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus saprophyticus* and *Staphylococcus aureus.* This result was analogous with the findings of Shashwati et al. [23].

Findings from the antibiotic sensitivity patterns of bacteria isolates in this study revealed that the level of resistance exhibited by implicated bacteria is very worrisome because resistant genes are being transferred to susceptible bacterial populations, and this will render commonly available antibiotics useless as observed in this study. Forty-two (87.5%) of the total 48 isolates from urine samples showed marked resistance to more than 3 to 4 classes of antibiotics and were classified as multidrug resistant. This notion was supported by Mirsoleymani et al. [24] who isolated mainly Enterobacteriaceae from urine samples most of which were multidrug resistant. Increased resistance to cephalosporins among Enterobacteriaceae in this study could be due to its excessive use in the hospice used as case study.

Quinolones including ciprofloxacin and ofloxacin are commonly recommended antibiotics to treat bacterial infections especially urinary tract infections. The chemotherapeutic-resistant rate against this class of antibiotics is also skyrocketing among the Enterobacteriaceae isolates as stated by Leski et al. [25], Segar et al. [26], Parajuli et al. [27]. Resistance to quinolones typically arises as a result of alterations in the marker enzymes and modification in drug admission and efflux as reported by Leski et al. [25], Parajuli et al. [27]. Pervasive use of fluoroquinolones has added to the speedy surfacing of resistance globally as opined by Livermore [28]. Over-the-counter availability of all these drugs and deficiency in pragmatic isolation of patient infected with MDR bacteria has led to wide application of these chemotherapeutics and spread of MDR bacteria in the teaching hospital setting used as case study which enlighten the soaring pace of resistance against these groups of antibiotics.

Multiple Antibiotics Resistant index ranges from 0.5 to 1.0 which is very high. MAR index of 0.2 or higher indicates elevated threat resource of contamination and plasmid-mediated resistance where antibiotics are frequently used (antibiotic usage abuse) which in turn confers high propensity and tendency for antibiotic resistance among the multidrug resistant bacterial isolates as supported by Andy and Okpo [29].

Molecular characterization of highly ciprofloxacin-resistant *Pseudomonas aeruginosa* strain mcbay 1 with GenBank accession number MT423976 and *Enterococcus faecalis* strain mcbay 2 with GenBank accession number MT423977 from urine samples has shown that qnrS2 and qnrB genes as supported by Mohammed et al. [30], Najafi Mosleh et al. [31] respectively are implicated as resistant genes in this study. *P. aeruginosa* and *E. faecalis* molecularly-identified as *P. aeruginosa* and *E. faecalis* isolates resistant to ciprofloxacin and fluoroquinolone may have been spreading in urine specimens of patients from University of Medical Sciences Teaching Hospital, Akure.

The reason for quinolone resistance in bio-cellularly identified *E. faecalis* could be due to the profusion of enterococcal species resistant to fluoroquinolone drugs. Multiple studies encompass a frightening intensity of fluoroquinolones-resistant *Enterococcus* species in Iran as supported by Fozouni et al. [32], whilst a small number of studies have demonstrated varying findings as detailed by Datta et al. [33]. This dissimilarity might be owed to geological set up and sanitized states in diverse hospice catchment region.

CONCLUSION

The occurrence of antibiotic resistant genes (ARGs) associated with ciprofloxacin-resistant *Pseudomonas aeruginosa* quinolone-resistant *Enterococcus faecalis* isolates in urine samples generated by the hospital in this study further confirms that multidrug resistant bacteria is one of the major leading bacterial consortia capable of causing nosocomial urinary tract infections in patients. This calls for over-the-counter regulation in the prescription and use of anti-pseudomonal and anti-enterococcal quinolones for supposed treatment of urinary tract bacterial infections in hospital settings as overuse and misuse of the common cephalosporins and quinolones in chemotherapeutics has now elicited ultra resistance overtime by the implicated bacteria.

Authors' Contributions: AOO and BOO designed the study. MTB developed the methodology and acquire the data, analyse the data and interpreted the data. MTB wrote the manuscript, AOO and BOO corrected and fine-tuned the manuscript. MTB reviewed and revised the manuscript and provided technical and material support. AOO and BOO provided administrative support and both aptly supervised the study. All authors read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

Ethical approval: Ethics consent was procured and approved by the Ondo State health research ethics committee (OSHREC) of the ministry of health, Akure, Ondo State, Nigeria. The ethics statement document carries a health research ethics committee assigned number of NHREC/18/08/2016 and a protocol number of OSHREC/16/09/2019/245.

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