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ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND PLASMID PROFILES OF BACTERIAL ISOLATES FROM FOUR HOSPITALS IN JOS METROPOLIS

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ABSTRACT

Antimicrobial resistance poses a serious threat to healthcare, leading to protracted illness, increased mortality risk, and spread of infection. The World Health Organization recommends continuous surveillance of antibiotics for resistance. This study aimed to investigate the pattern, scale, and possible mechanisms of resistance of common bacterial pathogens. A total of 174 bacterial isolates from biological samples (mainly urine and stool) were collected from four hospitals in Jos metropolis. The organisms were grown in selective media and characterized by colonial morphologies, Gram stain and biochemical tests. Antimicrobial sensitivity tests were performed using the disc diffusion method. Plasmid curing and β-lactamase test was conducted on multidrug resistant isolates using ethidium bromide as the curing agent in addition to gel electrophoresis of cured plasmids. Out of 174 isolates, 74 (42.5%) were *E. coli*, 48 (27.6%) *S. aureus*, 26 (14.9%) *Salmonella* species while 26 (14.9%) were *Streptococcus* species. Antimicrobial sensitivity test with 10 common antibiotics revealed 77 resistant phenotypes, with oxacillin and sulfamethoxazole/trimethoprim common in 55 (71.4%) and 60 (79%) of the phenotypes respectively, while oxacillin resistance had the highest (96-98%) occurrence. The activity of the antibiotics against the isolates revealed that gentamicin is the most active across all four facilities while oxacillin exhibited the lowest activity. The study revealed that Multiple-Antibiotic Resistance indices of 147 (84.5%) of isolates were greater than 0.2 while 34 (92%) of 37 selected isolates had β-lactamase enzymes. Treatment of the selected group with ethidium bromide revealed 13.5% cure rate while, gel electrophoresis revealed the presence of plasmids in 19 (51.3%) of the 37 selected isolates. This study shows that a high prevalence of multiple antibiotic resistance exists among common bacterial pathogens and is mediated by both chromosome and plasmids. The susceptibility profiles could be used to promote responsible antibiotic prescribing in these facilities.

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INTRODUCTION

One of the major challenges of anti-infective therapy is antimicrobial agents' resistance which was seen few years after the first effective antibiotics were developed and introduced into clinical use [1,2]. The resistance to many antibiotics, such as the

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aminoglycosides, fluoroquinolones has continued even with the carbapenems considered the main antibiotics active against Gram-negative bacteria [3]. Many studies have shown that the incidence of antimicrobial resistance (AMR) is higher with antibiotics regularly employed to treat common bacterial pathogens in communities, hospitals and agriculture [4,5]. Though a natural phenomenon, it is believed that this unrelenting exposure of antibiotics to the organism is mainly responsible for the selective pressure responsible for the high prevalence of resistant bacteria [6].

AMR is a global epidemic and the lack of new antibiotics to halt the menace has led experts to conclude we may be at the dawn of a post-antibiotic era [7]. One strategy the World Health Organization (WHO) has adopted to fight against antimicrobial resistance is the periodic surveillance of clinical isolates of microorganisms for susceptibility trends [8]. Drug resistance in bacteria is mediated by chromosomes and mobile genetic elements such as plasmids. Plasmid mediated resistance is carried on R-plasmids which can be disseminated to diverse population of microorganisms and regions causing global epidemics [9].

Traditionally, antimicrobial resistance surveillance involve large national and global studies performed on an ongoing basis to check incidences of bacterial infections and patterns of antimicrobial resistances, however, local surveillances of antibiotic resistance contribute vital information to guide antimicrobial prescribing practices at individual facilities [10]. Jos metropolis is a cosmopolitan city found in north central Nigeria. The city is home to many primary, secondary and tertiary healthcare facilities where different medications including antibiotics are being used to treat common bacterial infections. This study was undertaken to determine local antimicrobial resistance trends and plasmid profiles of bacterial isolates in selected facilities to serve as a tool to guide empirical treatments in these facilities [11].

MATERIALS AND METHODS

Sample Collection, Processing and Storage

A total of 174 clinical isolates (originally isolated from urine, stool, blood, sputum, semen, wound and urethral swab, endocervical swab and high vaginal swab) were collected from four healthcare facilities in Jos Plateau State, namely Plateau Specialist Hospital (PSSH), Dadin Kowa Comprehensive Medical Center (D/Kowa), Bingham University Teaching Hospital (BUTH) and Our Lady of Apostles Hospital (OLA) from June to December 2015. The isolates were transported to the Pharmaceutical Microbiology Laboratory of the University of Jos where they were purified by growing them in selective media and identified by standard microbiological methods [12]. Single colonies of pure isolates were inoculated onto sterile nutrient agar slants, incubated at 37 ˚C for 24 hours and then observed for growth. The slants containing the isolates were stored in the refrigerator until needed for further evaluation.

Permission

Clearance was obtained from the hospital facilities prior to commencement of study after presenting a letter, reference number **UHS/UJ/EC2015/vol.1/014**, granting ethical clearance for the study.

Antibiotic Susceptibility Testing of Purified Isolates

The modified Kirby-Bauer disc diffusion method was used as recommended by the Clinical Laboratory Standards Institute [13]. Overnight cultures of each bacterial isolate was standardized to 0.5 McFarland standard (1×10^8 CFU/mL) using sterile normal saline and used to flood sterile Muller-Hinton agar plates. The excess inoculum was drained off. Subsequently, the antibiotic sensitivity discs were aseptically placed on seeded plates, allowed to stand for 30 minutes and then incubated in an inverted position at 37˚C for 18 to 24 hours. Diameters of zones of inhibition were measured to the nearest milliliter using a transparent rule and results interpreted as sensitive, intermediate and resistant according to Hsueh *et al.* [13].

Calculation of Multiple Antibiotic Resistance Index

Multiple Antibiotic Resistance (MAR) index was calculated as a/b where “a” represents the number of antibiotics to which the isolates were resistant, and “b” represents the total number of antibiotics to which the isolate was exposed [14].

Plasmid Curing

Isolates that demonstrated resistance to more than four antibiotic agents were further subjected to plasmid curing using ethidium bromide. The Minimum Inhibitory Concentration (MIC) of ethidium bromide was determined using a 10 mg/ml stock solution after autoclaving at 121˚C for 15 minutes. Serial dilutions of the stock were made to obtain concentrations of 1.0, 0.5, 0.25, 0.125 and 0.01625 mg per mL in sterile nutrient broth, inoculated with 0.1 ml standardized inoculum and incubated at 37°C for 24 hours. The lowest dilution showing no visible

growth (turbidity) after incubation was considered the MIC. Subcultures were made from the tubes just above the MIC into fresh sterle broth and used to perform susceptibility tests against the panel of antibiotics.

Test for B-Lactamase Production

The iodometric method was used to detect the production of β-lactamase enzymes from the resistant bacterial isolates [15]. Five milliliter (5 ml) of an overnight culture of bacteria was added into 5 ml benzyl penicillin in 0.1ml of phosphate buffer of pH 6.0 and incubated for 30 minutes. Thereafter, 20 µl of 1% starch in distilled water was added to the suspension and 20 µl of 2% iodine in 53% potassium iodide. The activity of β-lactamase was indicated by decolorizations of iodine within 5-8 minutes. A negative control (bacterial suspension of each organism without the added reagents) was used to monitor the color change.

Extraction of Plasmid DNA

Plasmid analysis was performed on isolates that demonstrated resistance to more than 4 antibiotics. The procedure was performed at the molecular biology laboratory of the National Veterinary Research Institute Vom, Jos using the Zyppy™ Plasmid Miniprep Kit (Zymo Research) [16].

Data Analysis

Data were entered into Excel spreadsheet and diameters of inhibition zones classified according to CLSI interpretive chart as susceptible, intermediate and resistant [13]. For statistical analysis, intermediate susceptibilities were considered susceptible, resulting in dichotomous data as susceptible versus resistant and the results were recorded as frequencies and percentages. The analysis was performed using the MATLAB® software.

RESULTS

The demographic profile shows that of the 174 isolates more were obtained from females (128, 73.6%) than males (46, 26.4%); and more in adults (146, 84%) than children (28, 16%), (Table 1).

The distribution based on facility showed that the highest (47%) bacterial isolates were obtained from D/Kowa and the least from BUTH (Table 2).

According to source of the specimen, urine had the highest percentage of bacterial isolates (46%) whereas blood and urethral swabs had the lowest percentage (0.6%), (Table 3).

Escherichia coli was the most common bacterium identified in this study with a 42.5% occurrence while *Salmonella* spp. and *Streptococcus* spp. were the least common with 14.9% each (Table 4).

Figure 1 shows the percentage susceptibility profiles of the bacterial isolates in the study area. *E. coli* had good susceptibility to all the antibiotics except sulfamethoxazole/trimethoprim (SXT). Seven of the test antibiotics demonstrated good activity against *S. aureus*. The least active was oxacillin (OX). Resistance to oxacillin is often used as a marker for methicillin resistance. *Salmonella* spp. were susceptible to amoxicillin/clavulanic acid (AMC), vancomycin (VA), cefotaxime (CTX), gentamicin (GN), erythromycin (E), tetracycline (TE), ofloxacin (OFX) and ciprofloxacin (CIP) but resistant to SXT and OX. *Streptococcus* spp. were susceptible to VA, CTX and GN, but resistant to AMC, SXT, E, OX, TE and CIP.

Figure 2 shows percentage susceptibility of *E. coli* in the study area. Highest susceptibility to OX (100%) was observed at BUTH and PSSH while at D/Kowa it was to GN, OFX and CIP (96%, 80%, and 80%) respectively.

Figure 3 shows high susceptibility of *S. aureus* against GN (100%) at D/Kowa, GN (83%) at PSSH, AMC and GN (100%, 87%) respectively at OLA.

Figure 4 shows *Salmonella* species isolates as highly susceptible to VA, GN, E, TE, OFX, and CIP at OLA and at D/Kowa were susceptible to OFX (100%) and TE (96%).

Streptococcus spp shows 100 % susceptibility to VA, CTX, E, OFX and CIP at BUTH while at D/Kowa it was 80% susceptible to CTX and GN (Figure 5).

The Multiple Antibiotic Resistance Index (MARI) was determined as the number of antibiotics to which an organism was resistant. This was divided by the total number of antibiotics to which the organism was exposed.

The MARI for *E. coli*, *S. aureus*, *Salmonella* spp. and *Streptococcus* spp showed that majority of the isolates in all the four facilities had a MARI greater than 0.2 as shown in Tables 5a, b, c, d.

Table 6 represents B-lactamase profile of multidrug resistant isolates. The test revealed a high prevalence of the enzyme in the selected isolates. Of 37 selected isolates, only two (2) *E. coli* isolates (9%) were β-lactamase negative.

Figure 6 shows the gel electrophoresis of plasmids extracted from multidrug resistant isolates. Lane 1 is the DNA ladder (marker) used to identify the approximate sizes of the plasmids while the subsequent lanes represent the samples. It was observed that 19 (51%) of 37 selected isolates had plasmids as shown by the bright bands on the

electrophoresis plates. Most of the plasmids are of the 20 kb size, while some bacterial cells had only one type of plasmid. However, others had multiple plasmids of different sizes.

DISCUSSION

The demographic profile from the study showed that the incidence of bacterial infection was higher among females than males in all four facilities (Table 1). This could be because women visit hospitals more compared to men. From the data, adults were more affected by bacterial infections than children and a similar study by Mouton and Bazaldua [17] reported that the high incidence of bacterial infection in adults may be due to factors such as co-morbidities, increase in the number of invasive procedures and decreased physiological reserves. The predominance of the three species (*E. coli*, *Streptococcus* and *Salmonella* spp.) in D/Kowa could be due to the facility being the first point of contact for most patients in that community, so cases are diagnosed and treated while likely resistant cases are referred to secondary or tertiary care facilities (Table 2). The medical center is also located

Table 1: Demographic Profiles of Bacterial Isolates obtained from Records of Hospital Visits (N= 174)

Hospital	Female	Male	Adult	Children
D/Kowa	57	25	60	22
PSSH	40	6	44	2
OLA	20	9	25	4
BUTH	11	6	17	-
Total	**128**	**46**	**146**	**28**

Key: D/Kowa-Dadin Kowa Comprehensive Medical Center, PSSH- Plateau State Specialist Hospital, OLA-Our Lady of Apostles, BUTH- Bingham University Teaching Hospital

Table 2: The Distribution of Bacterial Isolates obtained from the Four Facilities

	Facility				
Bacterial Isolate	**D/Kowa**	**PSSH**	**OLA**	**BUTH**	**Total**
Escherichia coli	30	16	19	9	74
Staphylococcus aureus	4	30	8	6	48
***Salmonella* spp.**	24	-	2	-	26
***Streptococcus* spp.**	24	-	-	2	26
Total	**82**	**46**	**29**	**17**	**174**

Table 3: Distribution of Isolates according to their specimen of source

	Facility				
Specimen of Source	**D/Kowa**	**PSSH**	**OLA**	**BUTH**	**Total**
Urine	33	27	8	12	80
Stool	40	-	4	-	44
Sputum	3	-	3	1	7
HVS	6	13	5	1	25
Blood	-	1	-	-	1
Semen	-	2	-	2	4
Urethral Swab	-	-	-	1	1
Wound Swab	-	3	-	-	3
ECS	-	-	9	-	9
Total	**82**	**46**	**29**	**17**	**174**

Keys: HVS = High vaginal swab; ECS = Endocervical swab

Table 4: Isolate Types and their Distribution in Various Specimens

Specimen	Bacterial Isolate				
	E. coli	*S. aureus*	*Salmonella* spp.	*Streptococcus* spp.	Total
Urine	38	18	1	15	72
Stool	20	-	24	-	44
Sputum	-	4	-	6	10
HVS	6	17	1	2	26
Blood	-	1	-	3	4
Semen	1	3	-	-	4
Urethral swab	-	2	-	-	2
Wound swab	2	1	-	-	3
ECS	7	2	-	-	9
Total	**74 (42.5%)**	**48 (27.6%)**	**26 (14.9%)**	**26 (14.9%)**	**174**

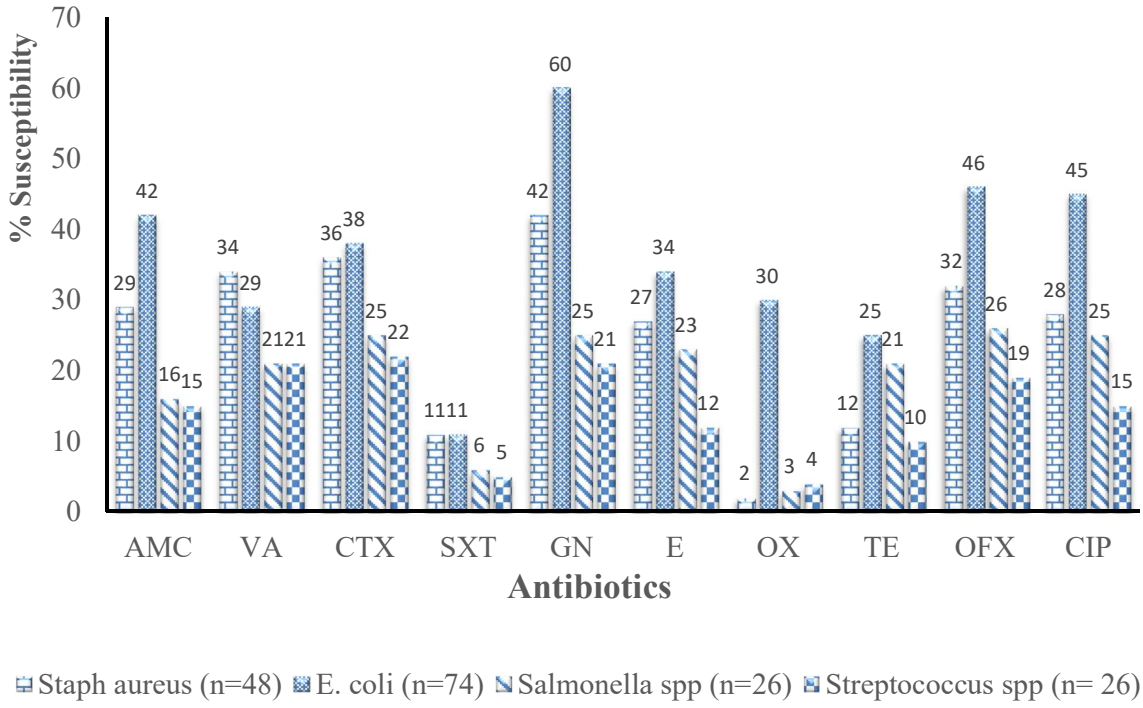

Figure 1: Susceptibility (%) of the Bacterial Isolates in the Study Area to test antibiotics
Antibiotic codes: AMC = Amoxicillin/Clavulanic acid ; VA= Vancomycin ; CTX= Cefotaxime ; SXT = Sulfamethoxazole/Trimethoprim (SXT); GN = Gentamycin; E = Erythromycin; OX = Oxacillin; TE = Tetracycline; OFX = Ofloxacin; CIP = Ciprofloxacin

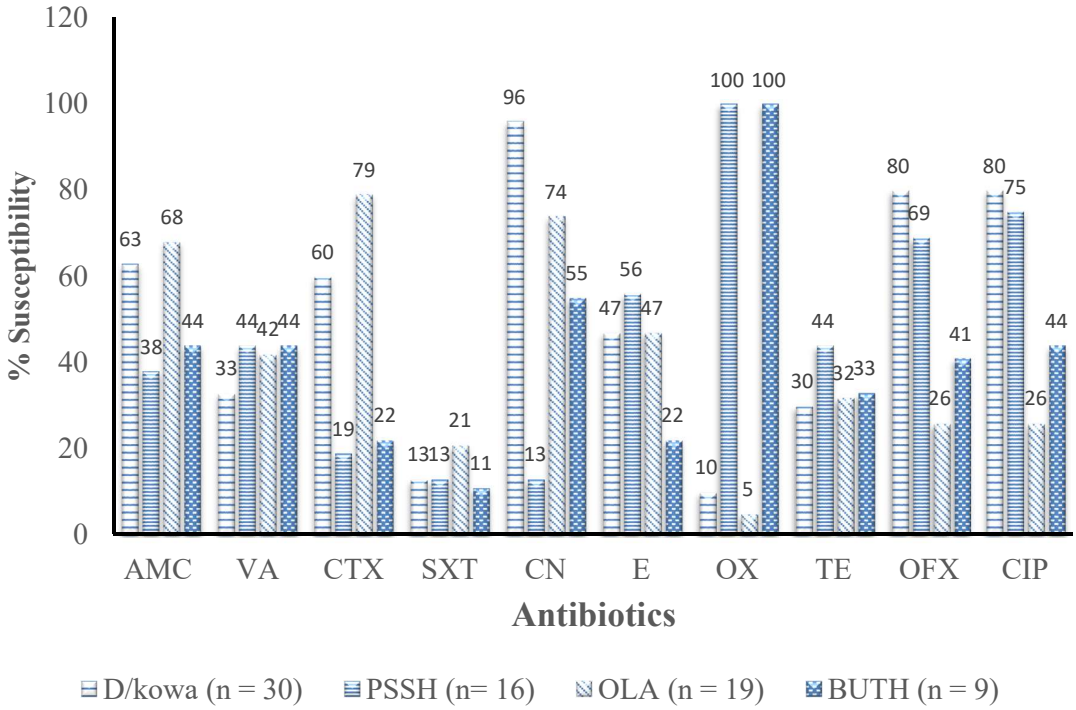

Figure 2: Susceptibility (%) of *E. coli* to test antibiotics in the Study Area

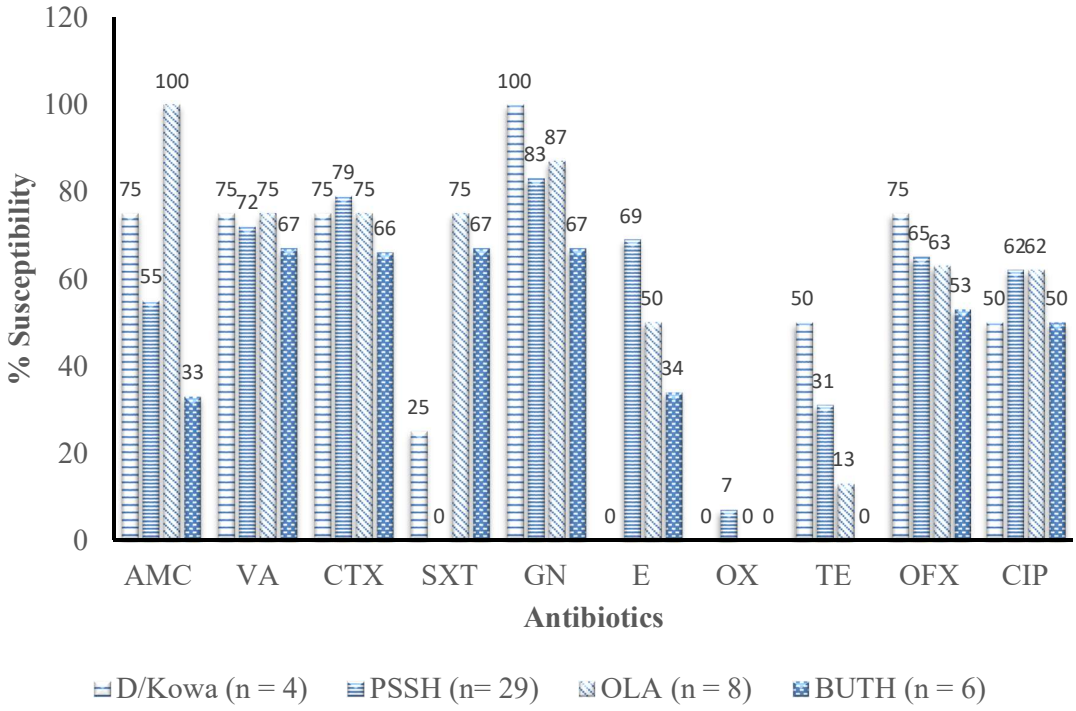

Figure 3: Susceptibility (%) of *S. aureus* to test antibiotics in the Study Area

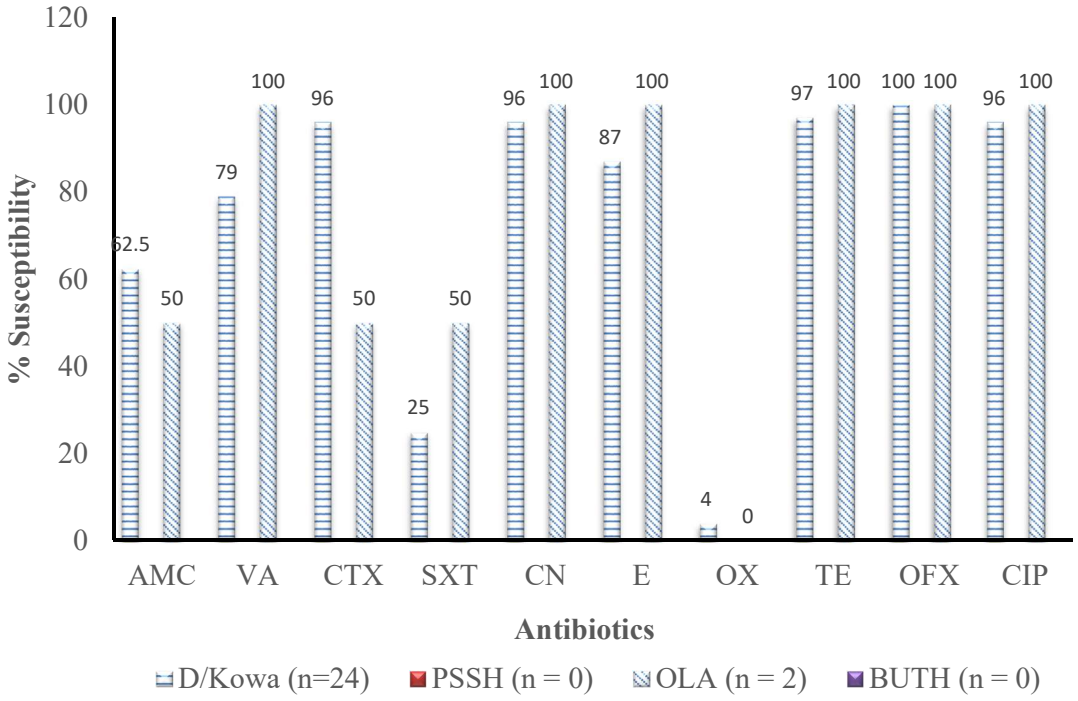

Figure 4: Susceptibility (%) of *Salmonella* species to test antibiotics in the Study Area

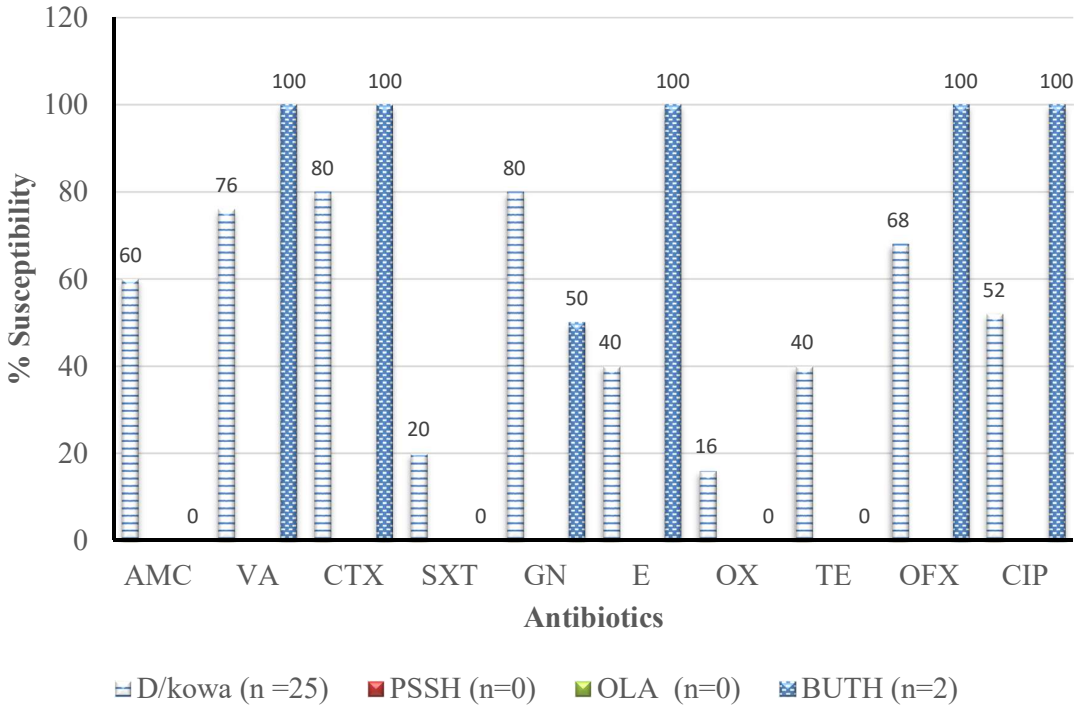

Figure 5: Susceptibility (%) of *Streptococcus* spp. to test antibiotics in the Study Area

Table 5a: Multiple Antibiotic Resistance Index (MARI) of *E. coli* across the facilities

MAR Index	No of isolates at given MARI			
	D/Kowa	PSSH	OLA	BUTH
	(n = 30)	(n =16)	(n =19)	(n =9)
0	-	-	-	-
0.1	-	2	1	1
0.2	4	1	1	-
0.3	5	1	1	-
0.4	11	1	3	3
0.5	3	3	4	-
0.6	3	2	4	-
0.7	2	-	2	-
0.8	1	1	1	3
0.9	1	-	2	1
1.0	-	5	1	1

Table 5b: Multiple Antibiotic Resistance Index of *S. aureus*

MAR Index	D/Kowa n = 4	PSSH n = 30	OLA n = 8	BUTH n = 6
0	-	-	-	-
0.1	1	-	-	-
0.2	-	-	-	-
0.3	1	9	2	1
0.4	1	5	3	2
0.5	-	12	1	1
0.6	1	1	-	-
0.7	-	1	1	-
0.8	-	-	1	-
0.9	-	2	-	1
1.0	-	-	-	1

Table 5c: Multiple Antibiotic Resistance Index of *Salmonella* spp.

MAR Index	D/Kowa	PSSH	OLA	BUTH
	n = 24	n = 0	n = 0	n = 2
0	1	-	-	-
0.1	1	-	-	-
0.2	6	-	-	-
0.3	12	-	-	-
0.4	1	-	-	-
0.5	2	-	-	-
0.6	1	-	-	-
0.7	-	-	-	-
0.8	-	-	-	-
0.9	-	-	-	-
1.0	-	-	-	-

Table 5d Multiple Antibiotic Resistance Index of *Streptococcus* spp.

MAR Index	D/Kowa	PSSH	OLA	BUTH
	n =25	n = 0	n = 0	n = 2
0	-	-	-	-
0.1	1	-	-	-
0.2	1	-	-	-
0.3	2	-	-	-
0.4	7	-	-	2
0.5	5	-	-	-
0.6	4	-	-	-
0.7	2	-	-	-
0.8	1	-	-	-
0.9	2	-	-	-
1.0	-	-	-	-

Table 6: B-lactamase Production of Multi-drug Resistant Isolates

Organism	Positive (%)	Negative (%)
***E. coli* (n = 23)**	21 (91)	2(9)
***S. aureus* (n =7)**	7 (100)	0 (0)
***Streptococcus* spp (n =5)**	5 (100)	0 (0)
***Salmonella* spp. (n =3)**	3 (100)	0 (0)

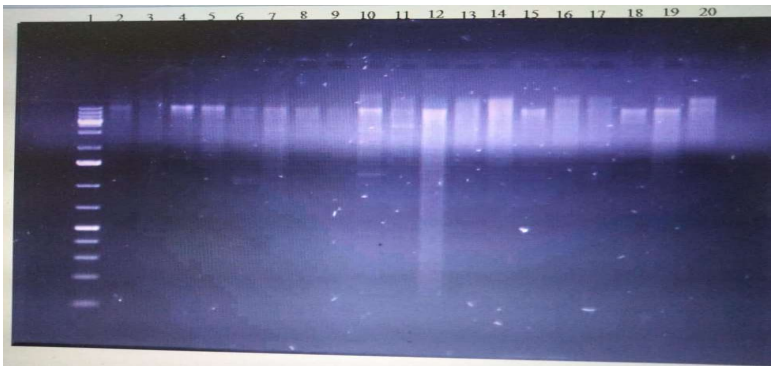

Figure 6: Plasmid Profile of Selected Bacterial Cells

in an overcrowded area which may account for poor infection control and the rapid spread of infections in the community. The high occurrence of *E. coli* may also be because most of these organisms were isolated from urine and stool (Tables 3 and 4), where they are frequently implicated in urinary tract infections (UTIs) and are part of normal gut flora [18]. On the other hand, blood, urethral swab and wound swabs showed little or no presence of bacteria. Besides *E. coli, S. aureus* was the second most frequently isolated organism from urine samples (Table 4). The prevalence of *S. aureus* in this study points to the rising implication of the organism as a urinary pathogen. Muder and co-workers [19] in their study reported the presence of *S. aureus* as a primary urinary pathogen among long-term-care patients. In our study, *S. aureus* was the most frequently isolated from PSSH, a teaching hospital and a referral center. The high occurrence of *Salmonella* (Figure 4) in D/Kowa agrees with what has been documented in literature that infections with this organism especially *Salmonella typhi* is endemic in tropical and subtropical regions of the world [20]. Physicians in this facility should therefore suspect Salmonellosis in patients that present with acute diarrhea or febrile illness without obvious cause.

The prominent level of activity of vancomycin against *S. aureus* in all four facilities shows that the drug can still be used in the treatment of many cases of staphylococcal infection even though the isolates have not been shown to be methicillin resistant. The average resistance rate of *S. aureus* to vancomycin across the four facilities is 28% (Figure 3). This may be noteworthy as Vancomycin Resistant *Staphylococus aureus* (VRSA) has been reported in literature to be on the rise globally due to increased use of the drug to treat MRSA strains [22]. The activity of oxacillin against *S. aureus* in the four facilities is seen to be the lowest of all 10 antibiotics assessed (Figure 1). This suggests that most of the *S. aureus* isolates are methicillin resistant as oxacillin is used as a marker for methicillin resistance. This agrees with what is already documented in literature that MRSA is widespread and is on the increase in both community and hospital settings [4, 23]. In D/Kowa *Streptococcus* isolates were most susceptible to gentamicin, cefotaxime and vancomycin while the agent of least activity was oxacillin, a β-lactam. This resistance may be mediated by β-lactamases. All the streptococcal isolates were β-lactamase producers (Table 6). In BUTH, the two *Streptococcus* isolates were completely susceptible to the fluoroquinolones, vancomycin, cefotaxime and erythromycin. These drugs should be considered in the antibiotic policy of this institution and treatment guidelines with respect to the treatment of pharyngitis and other infections suggestive of *Streptococcus*. *E. coli*, one of the Gram-negative organisms in the study was widely distributed among patients in all four facilities. Gentamicin, ofloxacin, and ciprofloxacin showed appreciable levels of activity against the organism in D/Kowa and PSSH (Figure 5). This agrees with work done by Liang and others [3] which revealed that aminoglycosides, the carbapenems and the fluoroquinolones are the antibiotics still effective against Gram-negative organisms.

Also seen in this study was the finding that the antibiotic of lowest activity against *E. coli* in all facilities was sulfamethoxazole/trimethoprim (SXT) (Figure 5). The availability and easy access to this antibiotic may account for the observation as there are reports that SXT is widely used in the developing world as first line treatment in many bacterial infections including UTIs and severe respiratory tract infections which has triggered over production of chromosomal Dihydrofolate reductase (DHFR) in *E. coli* [24, 25]. Dyar and co-workers [25] found a sensitivity rate of only 35% among 818 *E. coli*

isolates in children aged 6-60 months in Vietnam compared to the average of 10% across all facilities in this study. This difference may show a trend towards a higher prevalence of SXT resistant *E. coli*. The MAR indices of *E. coli* in all four facilities are comparable (Table 5a). The MAR index ranged from 0.1-1 meaning that none of the isolates was susceptible to all 10 antibiotics evaluated while one (1) isolate each from OLA and BUTH were resistant to all 10 antibiotics to which they were exposed (Table 5b). In all four facilities, more than 50% of *E. coli* was resistant to 4 or more antibiotics showing a high rate of multidrug resistance among this organism. *S. aureus* in this study is the most widely distributed being present in all specimens collected except from the stool (Table 4). The MARI of these isolates ranged from 0.1 to 1.0. (Table 5a-d).

Overall, 137 (79.7%) of all the isolates across the four facilities have an MAR index of >0.2. This suggests that these communities are high risk source of contamination where several antibiotics are often used [26]. The high MAR index in this study is also evidence in support of the findings published in literature that multi-drug resistant bacteria is widespread and on the increase [8]. Of the 38 isolates subjected to β-lactamase test, only 2 (5.3%) were β-lactamase negative. The majority (94.7%) were B-lactamase positive. The high occurrence of B-lactamase in multidrug resistant organisms revealed in this study agrees with the findings of Subhedar and Jain [27], who found that 93.4% of multi-drug resistant Gram-negative bacteria isolated from intensive care unit patients in a tertiary care facility in Central India were Extended Spectrum B-Lactamase (ESBL) though the test in this study did not indicate they were ESBL. Similarly, a high prevalence of ESBL was detected in Ibadan Southwest Nigeria by some researchers [28]. These findings may correlate with a trend towards increased prevalence of resistance to β-lactam antibiotics as already revealed by this study as well as the global spread of β-lactamase genes in the population [29]. This finding will therefore serve to sensitize prescribers about the risk of deploying β-lactam antibiotics for the empirical treatment of suspected bacterial infections. The findings on the prevalence of β-lactamases were based on phenotypic detection only therefore further investigations using molecular detection methods (genotypic techniques) will allow the typing of the β-lactamases into ESBL and other subtypes.

Plasmid curing test was performed to reveal the presence of plasmids in multiple antibiotic-resistant bacteria with ethidium bromide as the curing agent and the result revealed that the frequency of cured cells was 13.5% (with ethidium bromide concentration of 1000 to 16 µg/ml). Zaman and co-workers [30] found a cure rate of 14.29% with 100 µg/ml ethidium bromide with a frequency cure rate of 5.55% (with 50 µg/ml acridine orange), 7.4% (10% w/v sodium dodecyl sulfate), while Elias and co-workers [9] also revealed different cure rates when they employed different curing agents (ethidium bromide, sodium dodecyl sulfate, acridine orange and heat) on *P. aeruginosa* isolates. This suggests that the efficiency of plasmid curing varies with the curing agent and the conditions under which it was conducted. The cure rate may also be a function of plasmid copy numbers as it has been reported in literature that low copy number plasmid was efficiently cured by ethidium bromide [9]. This author further suggested that differences in DNA and RNA sensitivity are responsible for difference in ethidium bromide sensitivity to bacterial strains. However, the 13.5% cure rate achieved in this study agrees with that documented by Padilla *et al.* [31] who showed that the percentage of cured plasmids was not more than 20% in optimal conditions in *P. aeruginosa.* The absence of plasmid in some of the isolates suggests that antibiotics resistance by these organisms may be mediated via the chromosome [9].

LIMITATIONS OF THE STUDY

Plasmid-cured derivatives of the bacterial isolates which would have allowed direct comparison between the plasmid-containing and plasmid-cured cells could not be obtained. As a result, the DNA ladder was the only means of comparison used in the analysis.

CONCLUSION

A total of 174 bacterial were isolated from biological samples mainly urine and stool from four facilities namely D/Kowa, PSSH, OLA and BUTH. Seventy-four (74, 42.5%) were *E. coli*, 48 (27.6%) *S. aureus*, 26 (14.9%) *Salmonella* species while 26 (14.9%) were *Streptococcus* species. Antimicrobial sensitivity test with common antibiotics revealed 77 resistance phenotypes, with oxacillin and sulfamethoxazole/trimethoprim common in 55 (71.4%) and 60 (79%) of the phenotypes respectively, while ofloxacin resistance had the lowest occurrence. Antibiotic activity of the isolates revealed that gentamicin was the most active across all four facilities while oxacillin exhibited the lowest activity.

The study revealed that MAR indices of 147 (84.5%) of isolates were greater than 0.2 and that 34 (92%)

of 37 selected isolates for plasmid analysis produced β-lactamase enzymes. Treatment of the selected group with ethidium bromide revealed 13.5% cure rate while, gel electrophoresis revealed the presence of plasmids in 19 (51.3%) of the 37 selected isolates. This study showed a high occurrence of multiple antibiotic resistance among common bacterial pathogens and is mediated by both chromosome and plasmids. In conclusion, susceptibility profiles could be used to promote responsible antibiotic prescription in these facilities.

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