Correlation of Antibiotic Resistance with Some Virulence Properties Among Clinical Bacterial Isolates

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Abstract: In the last few decades, the levels of antibiotic resistant infections in the developing countries have increased steadily. This was as a result of combination of microbial characteristics and the selective pressure of antimicrobial use. In this study, 100 clinical samples were screened for bacterial genera. The identified organisms were tested for antibiotic resistant pattern, beta-lactamase production hemolytic activity and multiple antibiotic resistance index. Correlation of antibiotic resistance with tested virulent factors was determined by statistical means. Ninety two (92) bacterial isolates belonging to 9 genera were isolated from the 100 clinical samples. Of these isolates, 62(67%) were Gram negative while 30 (33%) were Gram positive. *E. coli* (25%) was the most isolated organism while the least were *K. pneumoniae* and *Shigella* spp with 2% each. All the organisms were mostly isolated from urine with the exception of *Salmonella* spp which occurred mostly in stool samples. Beta-lactamase was produced by 59(64%) of the isolated organisms, out of which 43(69.4%) and 16(53.3%) were Gram negative and Gram positive organisms respectively. None of the organisms was a-hemolytic, 64(69.6%) were y-hemolytic (non-hemolytic), while 28(30.4%) were P-hemolytic. The resistant pattern of all the isolates showed a multidrug resistant (MDR) phenotype especially among members of the family Enterobacteriaceae. On Gram negative organisms, the result showed significant correlation in antibiotic resistance between P-lac+ and P-lac- organisms (P=0.010) and between P-hemolytic and y-hemolytic organisms (P=0.001). On *Staphylococcus* spp, there was no significant correlation in antibiotic resistance between P-lac+ and P-lac- (P=0.401) and between P-hemolytic and y-hemolytic Staphylococcus spp (P=0.590).The high rate of antimicrobial resistance among bacterial isolates from common clinical specimens obtained from patients attending Mubi General Hospital, Adamawa State was rather alarming and required urgent attention.

Keywords: Correlation, antibiotic resistance, Virulence, P-lactamase, haemolytic, MDR

Introduction

The levels of antibiotic resistant infections in the developing world have increased steadily in the last few decades as a result of combination of microbial characteristics and the selective pressure of antimicrobial use (Blondeau and Tillotson, 2000). Microorganisms' mechanisms of overcoming the activities of antimicrobial agents include the production of structure-altering or inactivating enzymes (e.g. beta-lactamase or amino glycoside-modifying enzymes), alteration of penicillin-binding proteins or other cell-wall target sites, altered DNA gyrase targets, permeability mutations, active efflux and ribosomal modification (Aaterson, 2001; Levy, 2002). Multidrug-resistant bacteria in both the hospital and community environment are important concern to the clinician, as it is the major cause of failure in the treatment of infectious diseases, increased morbidity, and mortality and the evolution of new pathogens (Akinjogunla and Enabulele, 2010).

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Haemolysin provides clinical bacterial isolates with possible selective advantage by releasing iron from lysed erythrocytes and enhances pathogenicity by destroying phagocytic and epithelial cells (Naveen and Mathai, 2005). Haemolytic organisms are more likely to cause disease than non-haemolytic organisms (Grover *et al.*, 2013). One of the most commonly used and effective agent in the treatment of infectious diseases is the β-lactam antibiotics which constitute 60% of the global antibiotic usage (Livermore and Woodford, 2006). However, relentless use of β-lactam antibiotics in the clinical practice has resulted in the appearance of β-lactamases (Ho *et al.*, 2005; Akinjogunla and Enabulele, 2010) and newer β-lactamases (George *et al.*, 2014)such as extended spectrum β-lactamases (ESBLs), which are plasmid mediated and are seen in both Gram negative and Gram positive organisms. It was also reported that three out of the top six dangerous pathogens are β-lactam resistant bacteria (Boucher *et al.*, 2009). Therefore, this study examined multidrug resistance phenotype and the relationship between some virulent factors and antibiotic resistant phenotype among both Gram positive and Gram negative isolates.

Material and Methods

Sample collections

One hundred clinical samples classified into six groups were obtained from routine section in Microbiology Laboratory of General Hospital Mubi, Adamawa State, Nigeria. The sources of the samples were as follows: urine, wound swab, high vaginal swab (HVS), stool, semen and sputum. The samples were analysed for bacterial growth within 1-2 hrs after collection.

Identification of bacterial isolates

All the samples were inoculated on MacConkey agar, Blood agar and replicated on Mannitol salt agar (oxoid, England). The plates were incubated at 37°C for 24h. Identification of bacterial isolates was done on the basis of their cultural and standard biochemical characteristics (Washington *et al.*, 2006).

Determination of β - lactamase activity of isolated organisms

The clinical bacterial isolates were screened for β-lactamase activity by iodometric method (Sale *et al.*, 2008). Briefly, 0.1 ml of 25 mg/ml Penicillin G was dispensed into 12 test tubes. Heavy inoculum of each of the test organisms on Nutrient agar slants was emulsified in 12 separate test tubes containing 0.1 ml of 25 mg/ml penicillin G. A heavy growth of test organism was also added to a tube containing 0.1 ml of distilled water served as control. The test tubes were then incubated at room temperature (30°C) for 90 min. After which 2 drops of 3% starch solution were added to each test tube and mixed by gentle swirling. One drop of iodine solution was added to each of the test tubes and the tubes were then allowed to stand for 5 - 10 min. Disappearance of blue color within 5 - 10 min confirms the presence of - lactamase activity.

Detection of haemolysin production.

Haemolysin production was detected using the method described by Martinez-Martinez *et al.*, (1999). All bacterial isolates were grown on 5% blood agar at 37°C for 24hours.The presence of clear zone around the colonies was taken as positive for haemolysin production.

Antibiotic Susceptibility Testing

Isolates were tested for antimicrobial susceptibility testing by the standard disc diffusion method. Standard inoculums adjusted to 0.5 McFarland was swabbed on Nutrient agar (Hi Media Laboratories, Mumbai, India) and was allowed to soak for 5 minutes. After that antibiotic discs were placed on the surface of media and pressed gently. Nutrient agar plates were then incubated at 37°C for 24h. The degree of susceptibility of the test isolates to each antibiotic were measured and interpreted as either sensitive (S) or resistant (R) according to the recommendations of clinical and laboratory standards (CLSI, 2007). The following standard antibiotic discs were used for the isolates; perfloxacin (10µg), gentamycin (10µg), ampiclox (30µg), cefuroxime (20µg), amoxicillin (30µg), ceftriaxone (25µg), ciprofloxacin (10µg), streptomycin (30µg), cotrimoxazole (30µg) and erythromycin (10µg) for Gram positive bacterial isolates. While augmentin (25µg), gentamycin (10µg), perfloxacin (10µg), ofloxacin (30µg), streptomycin (30µg), cotrimoxazole (30µg), chloramphenicol (30µg), sparfloxacin (10µg), ciprofloxacin (10µg) and amoxicillin (30µg) were used for Gram negative organisms.

Determination of Multiple Antibiotic Resistance Index (MAR)

The multiple antibiotic resistance (MAR) index was determined using the formula MAR = x/y, where x was the number of antibiotics to which test isolate displayed resistance and y was the total number of antibiotics to which the test isolates has been evaluated for sensitivity (Krumpermann, 1983; Akinjogunla *et al.*, 2014).

Statistical Analysis

Non-Parametric Mann-Whitney statistics was used to determine the correlation of antibiotic resistance between β-lactamase positive (β-lac+) and β-lactamase negative (β-lac-) and also between β-hemolytic and γ-hemolytic Gram negative and Gram positive organisms. All statistical analyses were carried out using the SPSS 17.0 window based program. Significance difference and Non-significance difference was defined when $p \leq 0.05$ and $p > 0.05$ respectively.

Results

The result in Table 1 showed the prevalence of bacterial isolates from clinical samples. Ninety two (92) bacterial isolates belonging to 9 genera were isolated. Of these isolates, 62(67%) were Gram negative while 30 (33%) were Gram positive. *E. coli* (25%) was the most isolated organism while the least was *K. pneumoniae* (2%) and *Shigella* spp (2%). Among Gram positive organisms, only *S. aureus* (19%) and Coagulase negative Staphylococci (13%) were isolated. All the organisms were mostly isolated from urine with the exception of *Salmonella* spp which occurred mostly from stool samples.

The result of this study also showed that 59(64%) bacterial isolates were β-lactamase producers out of which 43(69.4%) and 16(53.3%) were Gram negative and Gram positive organisms respectively. None of the organisms was α-hemolytic, 64(69.6%) were γ-hemolytic (non-hemolytic), while 28(30.4%) were β-hemolytic out of which 21(33.8%) and 7(23.3%) were Gram negative and Gram positive organism respectively (Table 2).

The results in Table 3 and 4 showed the resistance pattern of Gram negative and Gram positive

isolates respectively. *Shigella* sp was resistant to all the tested antibiotics. *Providencia rettgeri* was also resistant to all the tested antibiotics except on ofloxacin (75%) and Streptomycin (17%). All Gram negative isolates were 100% resistant to amoxacillin-clavulanic acid except *E. coli* which showed 96% resistant. Resistant to all other class of antibiotics by all the isolates were variable.

The multiple antibiotic resistance (MAR) index of all the isolates was presented in Table 5. The MAR index for all the isolates was variable and ranges from 0.1 – 1.0. The results showed that more than 80% of the organisms exhibited multi-drug resistance (MDR) phenotype out of which 59(95.2%) are Gram negative and 15(50%)Gram positive. The results also revealed that 25(27.2%) organisms had MAR Index of 1.0 while 72(78.3%) organisms had MAR Index that ranged from 0.5 – 1.0.

Table 6 and 7 showed the correlation of antibiotic resistance in relation to β-lactamase and hemolysin production.

On Gram negative organisms, the result showed significant correlation in antibiotic resistance between β-lac+ and β-lac- organisms (P=0.010) and between β-hemolytic and γ-hemolytic organisms (P=0.001).

On *S. aureus*, there was no significant correlation in antibiotic resistance between β-lac+ and β-lac- *S. aureus* (P=0.401). Whereas the antibiotic resistance of β-hemolytic *S. aureus* was significantly higher than that of γ-hemolytic *S. aureus* (P=0.006).

For Coagulase negative Staphylococci (CoNS), there was no significant correlation in antibiotic resistance between β-lac+ and β-lac- (P=0.214) and between β-hemolytic and γ-hemolytic CoNS (P=0.590).

Table1: frequency and Distribution of Bacterial Isolates from Clinical Samples

Organisms	Urine	HVS	W. swab	Sputum	Semen	Stool	Total
E. coli	15(65)	3(13)	2(9)	0	0	3(13)	23(25)
Salmonella spp	4(40)	0	0	0	1(10)	5(50)	10(11)
P. mirabilis	4(100)	0	0	0	0	0	4(4)
C. freundii	3(60)	2(40)	0	0	0	0	5(5)
C. diversus	3(75)	1(25)	0	0	0	0	4(4)
Shigella spp	1(50)	1(50)	0	0	0	0	2(2)
P. rettgeri	12(100)	0	0	0	0	0	12(13)
K. pneumoniae	1(50)	0	0	1(50)	0	0	2(2)
S. aureus	7(39)	6(33)	5(28)	0	0	0	18(20)
CoNS	9(75)	3(25)	0	0	0	0	12(13)
TOTAL	**59(64)**	**16(17)**	**7(8)**	**1(1)**	**1(1)**	**8(9)**	**92(100)**

Legend: CoNS = Coagulase negative Staphylococci, W. Swab= wound swab, HVS= high vagina swab

Table 2: Frequency (%) of organisms reacting to β-lactamase and hemolysin production

SN	Organisms	No. Tested	β-lactamase production (%)		Haemolytic reactions (%)		
			β-lac+	β-lac-	A	B	γ
1.	*E. coli*	23	18 (78)	5(22)	0	7(30)	16(70)
2.	*Salmonella* spp	10	7 (70)	3(30)	0	7(70)	3(30)
3.	*P. mirabilis*	4	3(75)	1(25)	0	1(25)	3(75)
4.	*C. freundii*	5	2(40)	3(60)	0	0	5(100)
5.	*C. diversus*	4	3(75)	1(25)	0	1(25)	3(75)
6.	*Shigella* spp	2	0	2(100)	0	0	2(100)
7.	*P. rettgeri*	12	10(83)	2(17)	0	4(33)	8(67)
8.	*K. pneumoniae*	2	0	2(100)	0	1(50)	1(50)
9.	*S.aureus*	18	10(56)	8(44)	0	5(28)	13(72)
10.	CoNS	12	6(50)	6(50)	0	2(17)	10(83)
	TOTAL	92	59(64.1)	33(35.9)	0	28(30.4)	64(69.6)

Table 3: Antibiotic Resistance Pattern Of Gram Negative Isolate

LEGEND:

S/N	Organism	AMC	CN	PEF	OFX	S	COT	CH	SP	CPX	AM
1	*E. coli*	22(96)	19(83)	15(65)	13(57)	17(62)	23(100)	16(70)	15(65)	16(70)	17(62)
2	*Salmonella spp*	10(100)	9(90)	7(70)	8(80)	9(90)	5(50)	7(70)	7(70)	8(80)	9(90)
3	*P. mirabilis*	4(100)	1(25)	1(25)	1(25)	4(100)	4(100)	00	3(75)	3(75)	4(100)
4	*C. freundii*	5(100)	4(80)	4(80)	4(80)	4(80)	4(80)	4(80)	2(40)	1(20)	5(100)
5	*C. diversus*	4(100)	4(100)	3(75)	3(75)	2(50)	4(100)	3(75)	4(100)	4(100)	3(75)
6	*Shigella* spp	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)
7	*P. rettgeri*	12(100)	12(100)	12(100)	9(75)	2(17)	12(100)	12(100)	12(100)	12(100)	12(100)
8	*K. pneumoniae*	2(100)	2(100)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)

AMC= Amoxacillin-clavulanic acid (25µg), CN= Gentamycin (10µg), PEF=Pefloxacin (10µg), OFX =Ofloxacin (30µg), S= Streptomycin (30µg), COT=Cotrimoxazole (30µg), CH= Chloramphenicol, 30µg), SP= Sparfloxacin (10µg), CPX= Ciprofloxacin (10µg), AM= Amoxacillin ((30µg). Figures in tables represent number of organisms resistant to antibiotics. Figures in parenthesis represent percentage of organisms resistant to antibiotics.

Table 4: ANTIBIOTIC RESISTANCE PATTERN OF GRAM POSITIVE ISOLATE

Antibiotics	*S. aureus*	CoNS
Perfloxacin	10(56)	6(50)
Gentamycin	10(56)	9(75)
Ampiclox	13(72)	8(67)
Cefuroxime	9(50)	6(50)
Amoxicillin	18(100)	10(83)
Ceftriaxone	11(61)	5(42)
Ciprofloxacin	7(39)	4(33)
Streptomycin	6(33)	5(42)
Cotrimoxazole	14(78)	7(58)
Erythromycin	11(61)	6(50)

Table 5: MAR Index of isolates

Isolates	MAR INDEX (%)[a]									
	0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
E. coli	1(4.3)	0	1(4.3)	2(8.7)	4(17.4)	0	2(8.7)	0	4(17.4)	9(34.1)
Salmonella spp	0	0	1(10)	0	1(10)	0	1(10)	1(10)	3(30)	3(30)
P. mirabilis	0	0	0	0	0	3(75)	1(25)	0	0	0
C. freundii	0	1(20)	0	0	0	0	0	2(40)	1(20)	1(20)
C. diversus	0	0	0	0	0	1(25)	0	0	2(50)	1(25)
Shigella spp	0	0	0	0	0	0	0	0	0	2(100)
P. rettgeri	0	0	0	0	0	0	0	3(25)	7(58.3)	2(16.7)
K. pneumoniae	0	1(50)	0	0	0	0	0	0	0	1(50)
S. aureus	2(11.1)	2(11.1)	2(11.1)	2(11.1)	0	0	1(5.6)	2(11.1)	3(16.7)	4(22.2)
CoNS	2(16.7)	0	3(25)	0	1(8.3)	1(8.3)	1(8.3)	1(8.3)	1(8.3)	2(16.7)
TOTAL	**5(5.4)**	**4(4.3)**	**7(7.6)**	**4(4.3)**	**6(6.5)**	**5(5.4)**	**6(6.5)**	**9(9.8)**	**21(22.8)**	**25(27.2)**

S/N	ANTIBIOTICS	β-lactamase (%)		Hemolysis (%)	
		β-Lac+ (n=43)[a]	**β-Lac- (n=19)[a]**	**β(n=21)[b]**	**γ(n=41)[b]**
1.	AMC	42(98)	19(100)	21(100)	40(98)
2.	Gentamycin	35(81)	18(95)	19(90)	34(83)
3.	Perfloxacin	31(72)	13(68)	16(76)	29(71)
4.	Ofloxacin	29(67)	11(58)	15(71)	26(63)

5.	Streptomycin	27(63)	13(68)	12(57)	28(68)
6.	Cotrimoxazole	43(100)	16(84)	20(95)	39(95)
7.	Chloramphenicol	32(74)	11(58)	14(67)	26(63)
8.	Sparfloxacin	32(74)	14(74)	17(81)	29(71)
9.	Ciprofloxacin	34(79)	13(68)	17(81)	30(73)
10.	Amoxacillin	40(93)	14(74)	17(81)	36(88)

Table 6: resistance pattern of Gram negative isolates based on hemolysin and β-lactamase production
Legend: AMC= Amoxacillin-Clavulanic acid, **β-lac+** = B-lactamase positive organisms, **β-lac-** = B-lactamase negative organisms, β and γ = beta and gamma haemolytic organisms respectively.**a= significant correlation (P=0.010), b= significant correlation (P=0.001).**

Table 7: **Resistance pattern of Gram positive isolates based on hemolysin and B-lactamase production**

Antibiotics	*Staphylococcus aureus*				CoNS			
	β-lactamase (%)		Hemolysis (%)		β-lactamase (%)		Hemolysis (%)	
	B-lac+ (n=10)[a]	B-lac- (n=8)[a]	β(n=5)[b]	γ(n=13)[b]	B-lac+ (n=6)[c]	B-lac- (n=6)[c]	β(n=2)[d]	γ(n=10)[d]
PEF	10(100)	0	4(80)	6(46)	4(67)	2(33)	0	6(60)
CN	9(90)	1(13)	4(80)	6(46)	6(100)	3(50)	2(100)	7(70)
APX	10(100)	3(38)	5(100)	8(62)	4(67)	4(67)	0	8(80)
CXM	9(90)	0	3(60)	6(46)	4(67)	0	0	6(60)
AM	10(100)	8(80)	5(100)	13(100)	5(83)	5(83)	1(50)	9(90)
CTX	10(100)	1(13)	5(100)	6(46)	4(67)	1(17)	1(50)	4(40)
CPX	6(60)	1(13)	3(60)	4(31)	3(50)	0	0	4(40)
S	5(50)	1(13)	3(60)	4(31)	3(50)	2(33)	0	5(50)
COT	10(100)	4(50)	5(100)	9(69)	5(83)	2(33)	1(50)	6(60)
E	10(100)	1(13)	4(80)	7(54)	5(83)	1(17)	1(50)	5(50)

Legend: PEF= Perfloxacin, CN= gentamycin, APX= ampiclox, CXM= Cefuroxime, AM=Amoxicillin, CTX= Ceftriaxone, CPX= Ciprofloxacin, S= Streptomycin, COT= cotrimoxazole, E= Erythromycin, B-lac+ = B-lactamase positive, B-lac- = B-lactamase negative.
a= no significant correlation (P=0.401), b= significantly different (P=0.006), c= no significant correlation (P=0.214), d= no significant correlation (P=0.590)

Discussion

The finding that *E. coli* was the most abundant organism from most clinical samples was in agreement with previous studies (Oduyebo *et al*., 2015; Cristina *et al*., 2017). Our findings also showed that *P. mirabilis* and *P. rettgeri* were the most isolated bacterial organisms from urine. This was in contrast with previous studies (Kukanur *et al*., 2015; Ntirenganya *et al*., 2015) which reported that *E. coli* was the most isolated uropathogen.

The result of the present study also showed that Beta-lactamase enzyme was produced more by Gram negative isolates than Gram positive isolates. This was probably because beta-lactam antibiotic are the mostly prescribed drugs involving infections associated with most of the organisms under study. This observation was consistent with previous reports which raised an alarm over increasing rate of cephalosporin resistance by Gram negative bacterial isolates of hospital origin (Paul *et al*., 1997; Iyoha and Tula, 2014). Another report from Nigeria revealed that β-lactams are the most frequently prescribed antibiotics especially in Gram negative infections and selective pressure exerted by the use of these β-lactam drugs have resulted in the strains producing the extended spectrum β-lactamases enzymes (Aibinu *et al*., 2003).

There are four known ways of resistance to β-lactam antibiotics: i) production of β-lactamase enzymes that hydrolyse the β-lactam ring of the antibiotic, ii) penicillin binding proteins that maintain the peptidoglycan structure in bacterial cell wall, iii) alteration of porin channels, and iv) initiation of efflux exporter proteins (Fisher *et al*., 2005; Oztürk *et al*., 2015). Most *β*-lactamases contribute to resistance to a variety of antibiotics including the third- and fourth-generation cephalosporins and monobactams (Wei *et al*., 2005). Significant correlation in antibiotic resistance between β-lactamase positive and β-lactamase negative Gram negative organisms as shown in this study suggest that expression of the enzyme β-lactamase among them did not significantly contributes to the antibiotic resistance phenotype exhibited by these organisms. One of the factors that might lead to this observation might not be unconnected with the number of β-lactam antibiotics used in the panel of antibiotic. In this study, only two β-lactam antibiotics were used for Gram negative isolates.

In the present study, haemolytic activity was seen in 28(30.4%) of the total isolates. This finding was higher than 9% (Vaish *et al*., 2006) and 21% (Kausar *et al*., 2009) reported previously. In contrast to the findings of our study, higher haemolytic activities by pathogenic bacteria isolates were also documented. These include 54% (Desai et *al*., 2013) and 41.36% (Raksha *et al*., 2003) α-hemolysin production from their isolates. A study by SaiSwaroop *et al* (2013) has reported presence of 40% hemolysin production; all these findings were very much in contrast to our results. Contrary to our findings also, report from previous study showed that *Citrobacter freundii, Proteus vulgaris, Providencia* species, CoNS did not produce β-haemolysin (Egbe and Enabulele, 2014). The finding of our study showed that *Salmonella* spp (70%), *K. pneumoniae* (50%), *P. rettgeri* (33%), *E. coli* (30%), *S. aureus* (28%), *P. mirabilis* and *C. diversus* (25% each) and CoNS produced β-haemolysins variably. Another previous study (Priya *et al*., 2015) showed that none of their *E. coli* isolate produced either α or β haemolytic, which was also contrary to the findings of our study. Although none of the *E. coli* in our study are α-hemolytic. Similarly, lack of significant relationship between haemolysin production and antibiotic resistance among members of Enterobacteriaceae was not only peculiar to this study, but was previously reported among clinical bacterial isolates (Grude *et al*., 2008; Upadhyaya, *et al*., 2010; Egbe and Enabulele, 2014). In agreement with previous report (Martinez-Martinez *et al*., 1999), the finding of this study also showed that antibiotic resistance was more on non-hemolytic (γ-nemolytic) CoNS than haemolytic CoNS. Hemolysin has been suggested to increased virulence by increasing the availability of iron mediating toxic effect on leucocytes and other nucleated cells potentiating the effect on endotoxin, and mediating serum resistance (Egbe and Enabulele, 2014). Hemolysin, may also contribute to tissue injury to the survival of organism in renal parenchyma and entry into the bloodstream (Chen *et al*., 2006).

Our survey of antimicrobial resistance among bacterial isolates from common clinical specimens obtained from patients attending Mubi General Hospital, Adamawa State showed rather alarming rates of multidrug resistance phenotype among both Gram-negative and Gram-positive organisms. This was consistent with other studies from the sub-Saharan African region (Ntirenganya *et al*., 2015; Brink *et al*., 2008; Bentrand and Dowzicky, 2012).

All our Gram negative isolates were members of the family Enterobacteriaceae. The high multiple drug resistance (95.2%) phenotype exhibited by members of this family was rather alarming. This suggests high level of exposure to antibiotics which may result due to inappropriate or misuse of antibiotics in both community and hospital settings. The observed MDR among the Enterobacteriaceae family was not peculiar to our study alone. Previous studies have also reported high rate of MDR phenotype among this group of organisms (Ntirenganya *et al*., 2015; Muvunyi *et al*., 2011; Gangoue-Pieboji *et al*., 2006). Our study also showed that *S. aureus* are mostly resistant to antibiotics that are often used to treat staphylococcal infections. These include amoxicillin, cotrimoxazole, ampiclox, ceftriaxone, etc. This observation was similar to previous reports on the same organism (Dibua *et al*., 2014; Ntirenganya *et al*., 2015). This therefore suggests that this organism might have been acquired from the hospital settings and the resistant trait portrayed might

be due to selective pressure in the use of these antibiotics.

Conclusion

This study reported high rate of antibiotic resistance among clinical bacterial isolates which was demonstrated by their MAR index. Antimicrobial resistance is associated with high mortality rates and high medical costs and reduces the effectiveness of antimicrobial agents. MDR promotes the spread of resistant pathogens, which reduced efficacy of treatment, resulting in prolonged time of infection in patient and increased cost of treatment.

References

Aaterson, D.L. (2001). Extended-spectrum beta-lactamases: the European experience. *Curr. Opin. Infect. Dis.*, 14: 697-701.

Aibinu, I., Odugbemi, T., Mee, B.J. (2003). Extended spectrum β-lactamases in isolates of *Klebsiella* spp. and *Escherichia coli* from Lagos, Nigeria. *Nig. J. Health Biomed. Sci.*, 2(2): 53-60.

Akinjogunla, O.J., Ajayi, A.O.and Ekeh, N.O. (2014).Virulence factors and Antibiotic Resistant *Staphylococcus* spp from the Anterior Nares of Apparently Healthy Undergraduate Students in Uyo. *Amer. J. Res. Comm.*, 2(11): 158-180.

Akinjogunla, O.J., and Enabulele, O.I. (2010). Virulence factors, plasmid profiling and curing analysis of multi-drug resistant *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. isolated from patients with acute otitis media. *J. Am. Sci.* 6: 1022-1033.

Bertrand, X. and Dowzicky, M.J. (2012). Antimicrobial susceptibility among gram-negative isolates collected from intensive care units in North America, Europe, the Asia-Pacific Rim, Latin America, the Middle East, and Africa between 2004 and 2009 as part of the Tigecycline Evaluation and Surveillance Trial. *Clin. Ther.* 34: 124–137.

Blondeau, J. M. and Tillotson, G. S. (2000). Antimicrobial susceptibility patterns of respiratory pathogens- a global perspective. *Semin. Respir. Infect.*, 15:195-207.

Boucher, H.W., Talbot, G.H., Bradley, J.S., Edwards, J.E. and Gilbert D, et al. (2009) Bad bugs, no drugs: no eskape! an update from the Infectious Diseases Society of America. *Clin. Infect. Dis.*, 48: 1–12.

Brink, A., Feldman, C., Richards, G., Moolman, J. and Senekal, M. (2008). Emergence of extensive drug resistance (XDR) among Gram negative bacilli in South Africa looms nearer. *S. Afr. Med. J.* 98: 586–590.

Bush, K., Jacoby, G.A. and Medeiros, A.A. (1995). A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrob. Agents Chemother.* 39:1211-1233.

Chen, S.L., Hung, C.S., Xu, J., Reigstad, C.S, Magrini, V., Sabo, A. (2006). Identification of genes subject to positive selection in Uropathogenic strains of E.coli: a comparative genomics approach. *Proc. Natl. Acad. Sci. USA*, 103:5977-5982.

Clinical and Laboratory Standards Institute (CLSI). (2007). Document M100-S17.

Performance standards for antimicrobial susceptibility testing. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087–1898, USA.

Cristina, D., Paulina, P., Ionela, A., Nela, P., Lumini. T.T., Coralia, B., George, D.M.S.,

Mariana, C.C., Marinela, G. and Veronica, L. (2017). Antibiotic Resistance and Virulence Phenotypes of Recent Bacterial Strains Isolated from Urinary Tract Infections in Elderly Patients with Prostatic Disease. *Pathogens*, 6(22): 1-12.

Desai, S., Rajput, A., Kagal, A. and Bharadwaj R. (2013). Virulence factors in uropathogenic E.coli causing urinary tract infections. *Indian J. Basic Appl. Med. Res.*, 8(2):886-896.

Dibua, U.M., Onyemerela, I.S. and Nweze, E.I. (2014). Frequency, urinalysis and susceptibility profile of pathogens causing urinary tract infections in Enugu State, Southeast Nigeria. *Rev. Inst. Med. Trop. Sao Paulo*, 56: 55–59.

Egbe, C.A. and Enabulele, O.I. (2014). Haemolysin and Serum Resistance Profiles of Bacteria Isolates from Blood Culture. *Afr. J. Biomed. Res.* 17:203- 207.

Fisher, J.F., Meroueh, S.O. and Mobashery, S. (2005). Bacterial resistance to beta-lactam antibiotics: Compellingopportunism, compelling opportunity. Chemical Reviews 105: 395–424.

Foley, I.M. and Perrot, C.J. (1962) Screening of Bacterial Colonies for Penicilinase production. Nature 195:287-288.

Gangoue-Pieboji J, Koulla-Shiro S, Ngassam P, Adiogo D, Ndumbe P. (2006). Antimicrobial activity against gram negative bacilli from Yaounde Central Hospital, Cameroon. *Afr. Health Sci.*, 6: 232–235.

George, E.A., Sankar, S., Jesudasan, M.V., Sudandiradoss, C. and Nandagopal, B. (2014). Incidence of extended spectrum beta lactamase producing *Escherichiacoli* among patients, healthy individuals and in the environment. *Indian J. Med. Microbiol.*, 32(2): 172-174.

Grover, P.S., Bareja, R., Narang, V.K. and Jaryal, S.C. (2013). Incidence of fimbriated strains amongst haemolytic *Escherichiacoli*, International Organization of Scientific Research. *Journal Dent. Med. Sci.*, 4(3): 69-72.

Grude, N., Strand, L., Mykland, H., Nowrouzian, F. L., Nyhus, J., Jenkins, A. and Kristiansen, B. E. (2008). Fluoroquinolone-resistant uropathogenic *Escherichia coli* in Norway: evidence of clonal spread. *Clin. Microbiol. Infect.*, 14(5): 498-500.

Ho, P.L., Ho, A.Y.M., Chow, K.H., Wong, R.C.W., Duan, R.S., Ho, W.L., Mak, G.C., Tsang, K.W., Yam, W.C. and Yuen, K.Y. (2005). Occurrence and molecular analysis of extended-spectrum bet-

lactamase-producing *Proteusmirabilis* in Hong Kong, 1999-2002. *J.Antimicrob. Chemother.*, 55: 840-45.

Iyoha, O. and Tula, M.Y. (2014). Incidence and distribution of multi-drug resistant pathogens from clinical samples in a Tertiary Hospital in South-South Nigeria. *Afri. J. Clin. Experiment. Microbiol.*, 15 (3): 130-137.

Kausar, Y., Chunchanur, S.K., Nadagir, S.D., Halesh, L.H., Chandrasekhar, M.R. (2009).

Virulence factors, serotypes and antimicrobial suspectibility pattern of *Escherichiacoli* in urinary tract infections. *Al-Ameen J. Med. Sci.*, 2:47-51.

Krumpernam, P.H. (1983). Multiple antibiotic resistance indexing *E. coli* to identify risk sources of faecal contamination of foods. *Appl. Environ. Microbiol.* 46: 165- 170.

Kukanur, S., Meundi, M., Bajaj, A. and Kotigadde, S. (2015). Co-Relation between

Virulence Factors and Antibiotic Resistance of *E. coli*, With Special Reference to Uropathogenic *E. coli*. *IOSR J. Dental Med. Sci., 14(3)*: 15-21

Levy, S. B. (2002). Active efflux, a common mechanism for biocide and antibiotic resistance.

J. Appl. Microbiol., 92 Suppl: 65S-71S.

Livermore, D.M., Woodford, N. (2006) The beta-lactamase threat in enterobacteriaceae,

Pseudomonas and Acinetobacter. *Trends Microbiol.*, 14: 413–20.

Maztinez-Martinez, L, Fernandez, F. and Perea, E.J. (1999). Relationship between haemolysin production and resistance to fluroquinolones among clinical isolates of *Escherichia coli*. J. Anitimicrob. Chemother. 45: 277 – 279.

Muvunyi, C.M., Masaisa, F., Bayingana, C., Mutesa, L., Musemakweri, A., Muhirwa, G. and

Claeys, G.W. (2011). Decreased susceptibility to commonly used antimicrobial agents in bacterial pathogens isolated from urinary tract infections in Rwanda: need for new antimicrobial guidelines. *Am. J. Trop. Med. Hyg.*, 84: 923–928.

Naveen, R. and Mathai E. (2005). Some virulence characteristics of uropathogenic *Escherichiacoli* indifferent patient groups. *Indian J. Med. Res.*, 122, 2005, 143-147.

Ntirenganya, C., Manzi, O., Muvunyi, C.M. and Ogbuagu, O. (2015). High Prevalence of Antimicrobial Resistance among Common Bacterial Isolates in a Tertiary Healthcare Facility in Rwanda. *Am. J. Trop. Med. Hyg.*, 92(4): 865–870.

Öztürk, H., Ozkirimli, E. and Özgür, A. (2015). Classification of Beta-Lactamases and

Penicillin Binding Proteins Using Ligand-Centric Network Models. PLoS ONE 10(2): 1-23.

Oduyebo, O.O., Falayi, O.M., Oshun, P. and Ettu, A.O. (2015). Phenotypic determination of carbapenemase producing *Enterobacteriaceae* isolates from clinical specimens at a tertiary hospital in Lagos, Nigeria. *Niger Postgrad Med J.* 22(4):223-227.

Priya R., Kalaivani R. and Seetha K.S. (2015). Virulence Factors, Antimicrobial Resistance and Beta-Lactamase Production among Extra-Intestinal *E.coli* isolates from Various Clinical Samples. *Global J. Res. Analysis*, 4(5): 74-77.

Paul, S., Bezbaruah, M.K. and Gosh, A.C. (1997). Multiple antibiotic resistance (MAR) and its reversion in *Pseudomonas aeruginosa*. *Lett. Appl. Microbiol.*, 24: 169-171.

Raksha, R., Srinivasa, H. And Macaden, R.S. (2003). Occurrence and characterization of uropathogenic *Escherichiacoli* in urinary tract infections. *Indian J Med Microbiol.*, 21:102-107.

Sai Swaroop, P., HemaPrakashKumari P and SrinivasaRao U. (2013). Virulence-associated

factors in Escherichia coi strains isolated from urinary tract infections. *Int. J. Curr. Microbiol. App. Sci.* 2(10):436-440.

Sale, M., De, N., Doughari, J. H. and Pukuma, M. S. (2008). *In vitro* assessment of antibacterial activity of bark extracts of *Khaya senegalensis*. *Afr. J. Biotechnol.*, 7 (19): 3443-3446.

Upadhyaya, G.P.M., Umapathy, B.L., Ravikumar, K.L. (2010).Comparative Study for the

Presence of Enterococcal Virulence Factors Gelatinase, Hemolysin and Biofilm among Clinical and Commensal Isolates of *Enterococcus feacalis*. *J. Lab. Physic.*, 2(2): 100-104.

Vaish, R., Pradeep, M.S.S., Setty, C.R., Kandi, V. (2016). Evaluation of Virulence Factors and Antibiotic Sensitivity Pattern of *Escherichia coli* Isolated from Extraintestinal Infections. *Cureus* 8(5): 1-13.

Washington, C.W., Stephen, D.A., Williams, M.J., Elomer, W.K., Gary, W.P., Paul, C.S.and

Gail, LW. (2006). Koneman's Colour Atlas and Textbook of Diagnostic Microbiology, 6th edn. Lippincott Williams and Wilkins, Philadelphia, USA.

Wei, Z.Q., Chen, Y.G., Yu, Y.S., Lu, W.X. and Li, L.J. (2005). Nosocomial spread ofmulti-resistant *Klebsiella pneumoniae* containing a plasmid encoding multiple β-lactamases. *J. Med.Microbiol.*, 54(9): 885–888.

Yusuf, I., Arzai, A.H., Haruna, M.., Sharif, A.A. and Getso, M.I. (2014). Detection of multi drug resistant bacteria in major hospitals in Kano, North-West, Nigeria. *Braz. J. Microbiol.*, 45 (3): 791-798