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Original Research Article

Assessment of OqxA and OqxB Resistance Genes in *Escherichia coli* and *Klebsiella pnuemoniae* Clinical Isolates Based on Polymerase Chain Reaction from Niger Delta University Teaching Hospital, Yenagoa, Bayelsa State

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Abstract: Antimicrobial resistance continues to pose serious public health challenges. OqxA and OqxB are multidrug resistance genes that confer *Escherichia coli and Klebsiella pneumoniae resistance* to more than one antibiotics. The purpose of this study was to detect OqxA in *Escherichia coli* and *Oqx*B in *Klebsiella pneumoniae* from clinical samples isolated from Niger Delta University, Yenagoa. A total of 50 samples were collected. The bacterial isolates were identified using a standard bacteriological technique, the genes were detected using Polymerase Chain Reaction while the antibiotic susceptibility testing was done by disc diffusion. Of the 50 clinical isolates, 9(18%) were positive for *E. coli* while 15(30%) were positive for *Klebsiella pnuemoniae*. The total number of isolates were 18(36%) from male and 32(64%) from females. The susceptibility pattern of the isolates revealed that *Escherichia coli* exhibited the highest resistance of 100% to Cefuroxime and Augmentin, followed by Gentamycin and Ofloxacin (.55%) and Ciprofloxacin (11.11%) while *Klebsiella pneumoniae* Nalidixic Acid, Augmentin Cephalexin and Sulfamethoxazole shows 100% resistance respectively. Of the 9 that were E.coli isolates, 8(88.9%) harboured OqX A while of the 15 that were *Klebsiella pneumoniae* isolates, 13 (86.7%) harboured the OqxB genes. There was 100% resistance to Nalidixic acid, cefuroxime and sulfamethoxazole, with lowest resitance to meropenem (46.7%) and ciprofloxacin (60%). The report clearly demonstrates an urgent need for surveillance against these bacteria especially as they are pathogens of public health concerns to minimize the increasing pace of multidrug resistance conferred on these bacteria by OxAB genes.

Keywords: Genes, multidrug resistance, plasmid, PCR, Antibiotics, mutation, chromosome, Efflux pumps.

INTRODUCTION

Antimicrobial resistance continues to pose increasing challenge to public health (Fatima *et al*., 2021). The intrinsic and acquired resistance to antimicrobials can be demonstrated by the role of efflux pumps which were found in most bacterial species in advancing mutation accumulation site and reduction of antibiotic concentration intracellular. (Liu *et al*., 2020). Most of the efflux pumps are located on the chromosome of bacteria (Li *et al*., 2019). Plasmid-mediated efflux pumps have been described in recent years, such as QacBIII (Fatima *et al*., 2021), Tet(L) (Liu *et al*., 2020) and MexCD (Fu *et al*., 2018) efflux pumps. The OqxAB efflux pump belongs to the resistance nodulation division (RND) family and consists of OqxA as a periplasmic part and OqxB as a transmembrane protein (Abdullahi and Mustapha, 2020).

The oqxAB gene was first identified in 2003 on the pOLA52 plasmid in *Escherichia coli* from swine manure in Denmark (Abdullahi and Mustapha, 2020). The frequency of *oqxAB* of human oriented *E. coli* was discovered and reported in 2009 and significantly lower than that of the animal or environmental sources (Garza-Ranos *et al*., 2018). Since then, oqxAB has been increasingly detected among K. *pneumoniae* as one of the plasmid mediated

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quinolone resistance (PMQR) mechanisms over the past decades (Jun Li *et al*., 2019). The expression of the OqxAB efflux pump is regulated by RarA (regular of antibiotic resistance A) as an activator and OqxR (GntR-type transcriptional repressor) as a repressor. Transposition of oqxAB gene from chromosome to plasmid has the ability to increase the expression level of OqxAB efflux pump in more than 80-fold, leading to the expansion of the multidrug resistant phenotype (MDR) phenotypes (CLSI, 2020).

In recent studies, the *oqxAB* genes have been identified as the most prevalent plasmid mediated quinolone resistant (PMQR) genes in *E. coli* isolates from food-producing animals in China (Liu *et al*., 2020), as well as from animal-derived food products (Liu *et al.*, 2020). This could be due to the widespread use of olaquindox as a growth promoter for pigs weighing below 35 kg and mequindox against enteropathogenic *E. coli* infections in swine and poultry (Wang *et* al., 2017). Since K. *pneumoniae* is a hospital-associated pathogen that is continuously treated with multiple antibiotics, it developed resistance abilities through multiple mechanisms against most common antibiotics in clinical usage (Jun *et al*., 2019). This led to the emergence of multidrug resistant (MDR) *K. pneumoniae* that is responsible for high rates of morbidity and mortality due to the limited options of clinical treatment (Harada and Doi, 2018). Efflux pumps are one of the resistance mechanisms employed by *K. pneumoniae* to be involved in both intrinsic and acquired resistance to antibiotics by decreasing intracellular concentrations of antibiotics and promoting accumulation of mutations (Arato *et al*., 2021). The OqxAB is the predominant efflux pump found in *K. pneumoniae* that confers resistance against multiple antibiotics. (Abdullahi and Mustapha, 2020).

MATERIALS AND METHODS

A total of 50 samples were collected from Niger Delta University, Yenagoa. The bacterial isolates were identified using a standard bacteriological technique, the genes were detected using Polymerase Chain Reaction while the antibiotic susceptibility testing was done by disc diffusion.

RESULTS

Of the 50 clinical isolates, 9(18%) were positive for *E. coli* while 15(30%) were positive for *Klebsiella pnuemoniae*. The total number of isolates were 18(36%) from male and 32(64%) from females. The susceptibility pattern of the isolates revealed that *Escherichia coli* exhibited the highest resistance of 100% to Cefuroxime and Augmentin, followed by Gentamycin and Ofloxacin (.55%) and Ciprofloxacin (11.11%) while *Klebsiella pneumoniae* Nalidixic Acid, Augmentin Cephalexin and Sulfamethoxazole shows 100% resistance respectively. Of the 9 that were E.coli isolates, 8(88.9%) harboured OqX A while of the 15 that were *Klebsiella pneumoniae* isolates, 13 (86.7%) harboured the OqxB genes.

Table 1: Distribution of Specimen by Age (Male)

Age range	*Klebsiella pneumoniae*	*Pseudomonas Aeruginosa*	*Proteus Mirabillis*	*Citrobacter Freundii*	*Escherichia coli*	Total%
<20	4 (66.66%)	0	0	0	2 (33.33%)	6 (25%)
21-30	1 (50%)	1 (50%)	0	0	0	2 (8.33%)
31-40	1(14.28%)	2 (28.57%)	2 (28.57%)	2(28.57%)	0	7 (29.16%)
41-50	1 (20%)	1 (20%)	0	2 (40%)	1(20%)	5 (20.83%)
51-60	0	0	0	0	1 (100%)	1 (4.16%)
>60	1 (33.33)	1 (33.33%)	0	0	1 (33.33%)	3(12.5%)
Total	8 (33.33%)	5 (20.83%)	2 (8.33%)	4 (16.66%)	5 (20.83%)	24 (100%)

Table 2: Distribution of Specimen by Age (Female)

Age range	*Klebsiella pneumoniae*	*Peudomonas Aeruginosa*	*Proteus Mirabillis*	*Citrobacter freundii*	*Escherichia coli*	Total%
<20	4(44.44%)	0	1(11.11%)	1(11.11%)	3(33.33%)	9(34.62%)
21-30	0	1(33.33%)	2(66.66%)	0	0	3(11.54%)
31-40	1 (20%)	1 (20%)	1 (20%)	1 (20%)	1 (20%)	5(19.23%)
41-50	0	0	0	0	0	0
51-60	1 (100%)	0	0	0	0	1(3.85%)
>60	3(37.5%)	4 (50%)	1(12.5%)	0	0	8(30.76%)
Total	9(34.62%)	6 (25%)	5(20.83%)	2(8.33%)	4(16.66%)	26 (100%)

Table 3: Distribution of Specimen by Gender

SPECIMEN	MALE%	FEMALE%	TOTAL%
Urine	7(41.18%)	10(58.82%)	17(34%)
Sputum	4(80%)	1(20%)	5(10%)
Blood	3(60%)	2(40%)	5(10%)
Stool	6(75%)	2(25%)	8(16%)
Wound swab	4(50%)	4(50%)	8(16%)
High vaginal swab	0	7(100%)	7(14%)
Total	24(48%)	26(52%)	50(100%)

Table 4: Antimicrobial Susceptibility Testing

	GEN	CXM	OFL	AUG	NIT	CIP	MRP	S	CEP	NA	SXT
Isolates	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%
K. pneumoniae n=15	12(80%)	11(73.33%)	9(60%)	15(100%)	10(66.66%)	9(60%)	7(46.67%)	10(66.66%)	15(100%)	15(100%)	15(100%)
P. aeruginosa n=11	10(90.90%)	11(100%)	7(63.64%)	11(100%)	10(90.90%)	4(36.37%)	8(72.72%)	8(72.72%)	10(90.90%)	11(100%)	11(100%)
P. mirabillis n=7	5(71.45%)	7(100%)	4(57.14%)	7(100%)	6(85.71%)	5(71.45%)	2(28.59%)	7(100%)	7(100%)	7(100%)	7(100%)
C. freundii n=8	8(100%)	7(87.5%)	5(62.5%)	8(100%)	8(100%)	6(75%)	7(87.5%)	7(87.5%)	8(100%)	8(100%)	7(87.5%)
E .coli n=9	5(55.55%)	9(100%)	5(55.55%)	9(100%)	0	1(11.11%)	0	0	0	0	0
TOTAL 50	40(80%)	45(90%)	30(60%)	50(10%)	34(68%)	25(50%)	24(48%)	32(64%)	40(80%)	41(82%)	40(80%)

Keys: GEN: GENTAMICIN, CXM: CEPHALEXIN, OFL: OFLOXACIN, AUG: AUGMENTIN, NIT: NITROFURANTOIN, CIP: CIPROFLOXACIN, MRP: MEROPENEM, S: STREPTOMYCIN, CEP: CEFUROXINE, NA: NALIDIXIC ACID, SXT: SULFAMETHOXAZOLE

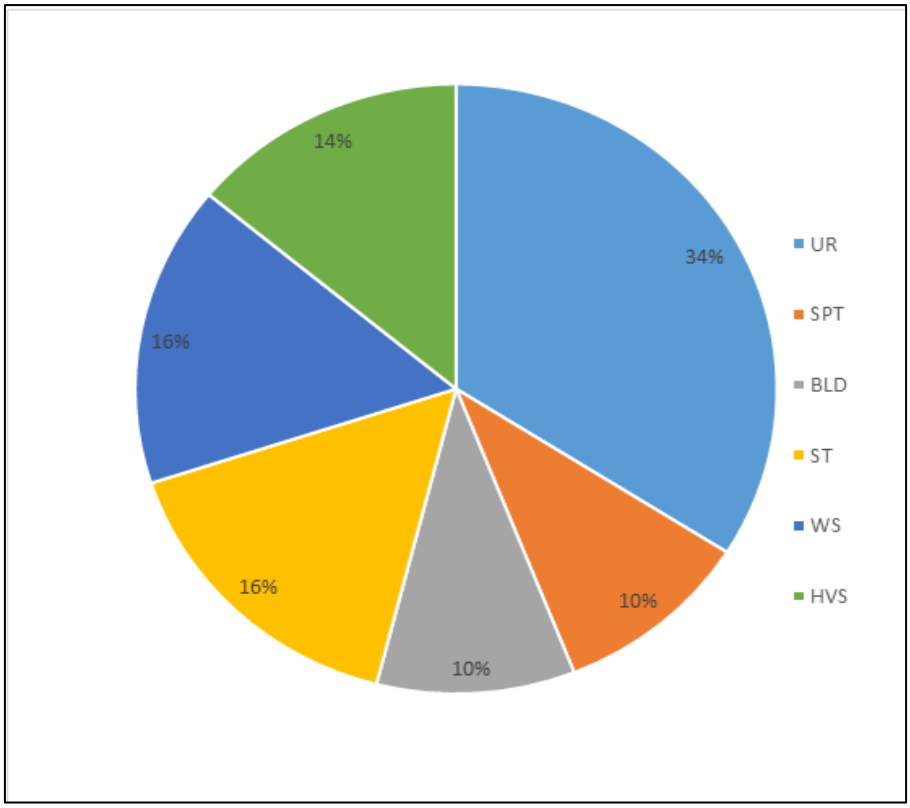


Fig. 1: Pie Chart Representing Sample Distribution
Stool =8, Sputum=5, Blood=5, High Vaginal Swab=7, Wound swab=8, Urine=17

Table 5: Distribution of Bacterial Isolates by Specimen

Specimen	***Klebsiella pneumoniae%***	***Pseudomonas aeruginosa%***	***Citrobacter freundii%***	***Escherichia coli%***	***Proteus mirabillis***	**Total**
Urine	6(35.29%)	1(5.88%)	7(41.18%)	1(5.88%)	2(11.76%)	17(34%)
Sputum	0	4(80%)	0	1(20%)	0	5(10%)
Blood	3(60%)	1(20%)	0	1(20%)	0	5(10%)
Stool	0	1(50%)	0	0	1(50%)	2(4%)
Wound swab	1(12.5%)	2(25%)	0	3(37.5%)	2(25%)	8(16%)
High vaginal swab	5(38.46%)	2(15.38%)	1(7.69%)	3(23.07%)	2(15.38%)	13(26%)
Total	15(30%)	11(22%)	8(16%)	9(18%)	7(14%)	50(100%)

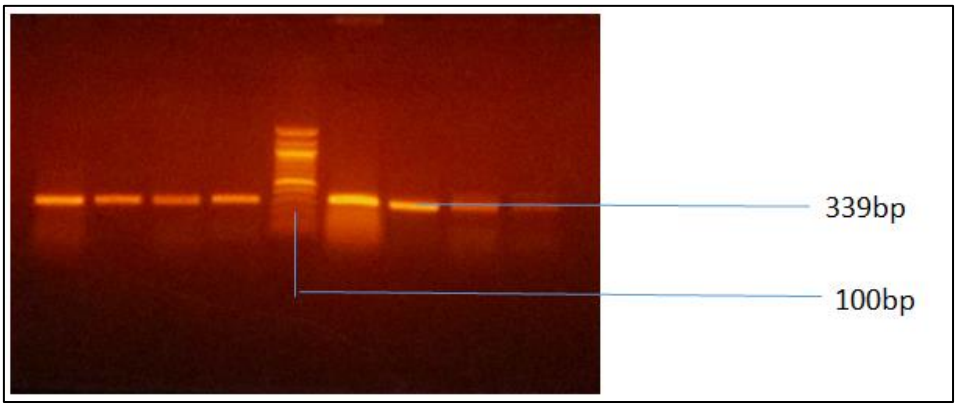
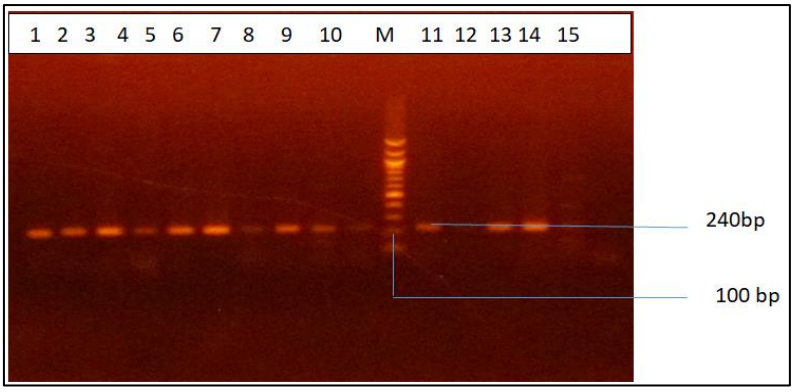


Plate 1: Agarose gel electrophoresis of OQXA gene of bacterial isolates. Lane 1, 2, 3, 4, 5, 6, 7, 8 and 9 represents the OQXA gene bands (339bp). Lane M represents the 100bp Molecular ladder.



DISCUSSION

Esherichia coli and *Klebsiella pnumoniae* are two among many clinical pathogens regularly implicated in urinary tract infections, which renews the interest in the study of OqxAB multidrug efflux pump genes. The occurrence of bacterial isolates with respect to age showed that *Klebsiella pneumoniae* were highest in females 9(34.62%) than males 8(33.33%). This may be due to the fact that females are more likely to have urinary tract infections which is often caused by *Klebsiella pneumoniae*. In *Klebsiella pneumoniae*, the age range <20 is observed to be highest for both male and female due to their high level of sexual activities which agrees with the reports of Clement at al (2020) on the inceasing threat to public health by *K.pnumoniae.*

The high occurrence in females may be due to the difference in urogenital anatomical structure whereby the urethra in female is shorter than that of the male which allows easy access of microorganism into the body. This may be due to the close proximity of the urethra and vagina to the anal canal and during clean up after defecation, organisms can be introduced into the vagina leading to infection which is in agreement with Nielubowicz, 2016.

Urine samples were the most predominant samples obtained from females in hospital cases, about 10(58.82%) and may be the cause of frequent urinary tract infections in hospitals in alignment with study. The clinical samples shows that out of 50 bacterial isolates of which 15(30)% were *Klebsiella pneumoniae* were obtained from urine, sputum, high vaginal swab, blood, and stool and wound swab indicates that *Klebsiella pneumoniae* are major pathogens of humans and usually causes urinary tract infections.

The overall antimicrobial resistance pattern of *Klebsiella pneumoniae* showed total resistance (100%) to Augmentin, Cefuroxime, Nalidixic acid and Sulfamethoxazole followed by 12(80%) Gentamicin, 11 (73.33%) Ceporex (cephalexin), 10(66.66%) Nitrofurantoin, 10(66.66%) Streptomycin, 9(60%) Ciprofloxacin, 9(60%) Ofloxacin, and, 7(46.67%) Meropenem. Nalidixic acid is the only quinolone drug that showed complete resistance. Other quinolone drugs that showed resistance were Ofloxacin 9(60%) and 9(60%) Ciprofloxacin. The only antibiotic that showed low antimicrobial resistance was Meropenem 7(46.67%) which may be due to the fact that *Klebsiella pneumoniae* is not often resistant to carbapenems.

This study reveals high prevalence of OqxA genes in the *E. coli* isolates and OqxB genes in *K. pneumoniae* isolates, which showed E.coli isolates, 8(88.9%) harboured OqX A while of the 15 that were *Klebsiella pneumoniae* isolates, 13(86.7%) harboured the OqxB genes. This agrees with earlier reports of Jinyi *et al.,* (2020) which reported that *oqxAB* was previously found in 74% of *K. pneumoniae* clinical isolates from South Korea, but *oqxAB* was detected in 100% of *K. pneumoniae* isolates in their study in China. The high prevalence of these MDR genes in isolates of clinical significance marks off a need for further clinical correlation of incidences of OqXAB genes and transference of resistance.

The antibiotic susceptibility profile further reveals multidrug resistance to quinolones and fluoroquinolones, such that renewed attention should be given to these bacteria especially, *K.pnumoniae* that is implicated in various nosocomial infections. And which increases morbidity and mortality rates. Thus, it could be well understood in this study that there is high incidence of OqxA in *E.coli* and OqxB in *K. pneumoniae* in this locality.

CONCLUSION

The prevalence of these highly resistant OqxA and B genes in *Escherichia coli* and *Klebsiella pnuemoniae* contributes significantly to prolonged hospitalization and increased mortality. Greater efforts need to be taken to explore pathways of resistance to 'last-resort' antimicrobials, especially among clinically relevant pathogens.

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