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Transformation of Extended Spectrum Beta lactamases (ESBLs) and *Sul* 1 genes obtained from trimethoprim-sulfamethoxazole resistant (TSR) *Escherichia coli* and *Pseudomonas aeruginosa* clinical isolates

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ABSTRACT

Background: Plasmid mediated Multi-Drug Resistant (MDR) *Sul*1 and ESBL resistance encoding genes are the major threats due to their ability to be transferred horizontally in any environment. In this study, the prevalence of *Sul*1 gene and ESBL coding genes (*blaCTX-M*, *blaTEM* or *blaSHV*) in *E. coli* and *P. aeruginosa* and determined their ability to transfer resistance through transformation. **Methods**: Ninety-two trimethoprim-sulfamethoxazole resistant TSR isolates belonging to *E. coli* and *P. aeruginosa* species were obtained from clinical samples such as urine, wound, and blood from patients in 3 government hospitals in Delta State, Nigeria. Resistance to antimicrobial agents was determined by disc diffusion methods. PCR amplification was performed on extracted plasmid DNAs for the detection of ESBL and Sul1 genes using specific primers. Extracted plasmid DNAs of ESBL producing and Sul1 positive isolates were used in transforming a competent *E. coli* K-12 adopting $CaCl_2$ method. **Results**: All 92 TSR isolates were MDR with a vast majority of resistant patterns associated with the cephalosporins, amoxicillin-clavulanic acid, and the fluoroquinolones. Sul1 gene and ESBL genotypes were produced in 29.3% of *E. coli* and 58.7% of *P. aeruginosa* isolates, respectively. *E. coli* was more prevalent ESBL producer (75.9%) than *P. aeruginosa* (24.1%) and the *blaCTX-M* was the most prevalent gene (30.4%). **Conclusion**: Twenty-three isolates transferred several antibiotic resistances which includes ceftazidime, cefotaxime, cefuroxime, cefixime, gentamicin, ciprofloxacin, ofloxacin trimethoprim-sulfamethoxazole and amoxicillin-clavulanic acid, Sul1 gene and ESBL genotypes by transformation, thereby indicating a high potential for dissemination of resistance markers in hospitals. These findings are of health concern because of the rise in antimicrobial resistance associated with ESBL isolates.

Introduction

Gross abuse in the use of beta lactam antibiotics is the leading cause of an ever increasing emergence of Extended Spectrum Beta Lactamases (ESBLs), which represent a major problem in the management of clinical infections. ESBLs which

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confer resistance to many antibiotics are detected in several members of the Enterobacteriaceae and are encoded by different genes inserted in mobile genetic element spread between bacterial species. Similarly, sulfonamide resistance in Gram-negative bacteria generally arises from the acquisition of Sul1 genes encoding dihydropteroate synthase [1]. The Sul gene is normally found linked to other resistance genes in class 1 integrons, making them important in the accumulation and transmission of multidrug resistance [2].

The three-primary mechanism of horizontal gene transfer (HGT) in bacteria are conjugation, transduction, and transformation contribute to the spread of antibiotic resistance. Of the three mechanisms of HGT, conjugation is the most studied in clinical environment [3-5]. Transformation is dependent on recipient cells that are competent to take up extracellular naked DNA. Generally, *E. coli* and *P. aeruginosa* are not believed to be naturally transformable., however, some reports indicate that these organisms can express modest competence under certain conditions that are feasible in its natural environments [6, 7].

Mobile genetic elements such as plasmids, integrons, and transposons have been identified as sources of multi-drug resistance in clinical isolates [8, 9]. Class I integrons are predominantly found in clinical isolates belonging to Gram-negative bacterial species [10]. The role of plasmids in conjugation mediated transfer of antibiotic resistance genes has been reported in numerous studies in Nigeria [11-13]. However, no study has reported the successful transfer of Sul1 genes and ESBLs in multi-drug resistant clinical isolates via transformation in Nigeria. Investigation in Sul1 genes and ESBLs are important as sulfonamides and beta lactam drugs are the most commonly prescribed drugs in many hospitals in Nigerian. The present study was therefore aimed at determining the prevalence of *Sul*1 gene and ESBLs in clinical isolates from urine, wound, and blood and to detect transformation-based HGT using *E. coli* and *P. aeruginosa* as donors.

Methods

Bacteria isolates selected for the study

A total of 368 E. coli and P. aeruginosa were isolated from urine, wound, and blood of patients admitted in 3 Government owned hospitals (Warri, Agbor and Asaba) in Delta state, Nigeria. The isolates were identified using Gram staining and biochemical tests following Clinical Laboratory Standards Institute (CLSI) procedure [14]. Antimicrobial resistance pattern were determined by the agar disc diffusion method on Mueller Hinton agar (Oxoid, England) using ten antimicrobial agents; ceftazidime (CZ-30µg) cefotaxime (CF-30µg) cefuroxime (CF-30µg), cefixime (CX-30µg), gentamicin (G-10µg), ciprofloxacin (C-5µg), ofloxacin (O-5µg) trimethoprim-sulfamethoxazole (T-25µg), amoxicillin-clavulanic acid (A-30µg), and nitrofurantoin (N-200µg) (Abtek, England) and results interpreted following the CLSI guidelines [14] with all intermediate resistances taken as resistant isolates.

Of the isolates, a total of 92 non-duplicate isolates of *Escherichia coli* (64) and *Pseudomonas aeruginosa* (28) which were resistant to trimethoprim-sulfamethoxazole and to at least 2 other different classes of antimicrobials used in the study (multi-drug resistant) was selected for further study.

Screening for ESBL by phenotypic test

ESBL production by phenotypic based diffusion test of double disc synergy test (DDST) was carried out on the selected isolates. Briefly, 1µL of the bacterial suspension prepared in sterile saline to a density equivalent to 0.5 McFarland turbidity standard were inoculated onto Mueller Hinton agar (MHA) plate and the inoculum was spread evenly with a swab. Plates were dried at room temperature, then cefotaxime (30µg) and ceftazidime disc (30µg) were applied 15 mm apart from the center of an amoxicillin-clavulanic acid (20µg/10µg) disc. Zones of inhibition around the cefotaxime and ceftazidime disc were measured after 24 hours incubation at 37ºC. Extension of zone of inhibition by ≥ 5mm towards amoxicillin-clavulanic acid was interpreted as ESBL producer.

Plasmid curing

Plasmid curing was carried out on the selected isolate using Sodium Dodecyl Sulphate (SDS) as curing agent. Overnight culture of *E. coli* and *P. aeruginosa* were used separately to inoculate broths containing serial dilutions of SDS and incubated at 37ºC for 24 hrs. Cultures showing no growth were discarded and the sub-inhibitory concentration was thus determined. A loopful of the sub-inhibitory concentration was streaked on MacConkey agar to obtain discrete colonies. Antibiotic sensitivity test was carried out on

colonies from the curing agent by disc diffusion. Isolates that lost their resistance phenotype after curing were interpreted as cured isolate.

Plasmid extraction and molecular detection of ESBL and Sul1 genes

Plasmid DNA extraction as described previously by Birnboim [15] was performed and resultant plasmids were resolved in a 0.8% agarose gel electrophoresis at 70V for 3 hours in Tris-acetic acid-EDTA buffer. After staining the gel with ethidium bromide, extracted plasmids were examined in a UV transilluminator and λ DNA ladder of various sizes were used as molecular weight markers (Bioneer Accupower, Korea).

Sulfonamide resistance gene (*Sul*1) and beta lactamases genes (*blaCTX-M, blaTEM* or *blaSHV*) were detected by PCR. Amplification was carried out using previously established primers listed in (Table 1).

Amplification of beta lactamases genes was performed in a volume of 25µL containing 1 X reaction buffer, 1.5mM $MgCl_2$, 200µm, dNTP,s 0.2µm each primer and 2.5 U of Taq polymerase (Eppendorf, Germany). Amplification conditions were as follow: initial heating block at 95^oC for 5 minutes followed by 35 cycles of denaturation for 30 sec s at 94^oC, 1 minute of annealing at 56^oC, extension at 72^oC for 1 minute and the final extension at 72^oC for 10 minutes

The PCR products were prepared and run on agarose as described above for plasmids.

Sul1 genes was amplified in a total volume of 25µL containing 2.5µL 10 X PCR buffer, 1 µL 50 mM $MgCl_2$, 2.5 µL 2 mM dNTP's ,0.15mM Taq polymerase, 2µL of DNA template and 0.5 µL primers. The thermal cycler (PTC-200, Germany) condition for amplification of Sul1 gene was 95^oC for 5 min, followed by 35 cycles of 94^oC for 30 sec, 53^{oC} for 30 sec and 72^oC for 1 min. A final extension of 5 min at 72^oC was performed.

At the end of each PCR, amplified products were separated by agarose (0.8%) gel electrophoresis and viewed with a UV transilluminator. Isolates harboring the Sul1 gene were selected for transformation study.

Transformation of *E. coli* k-12 with isolated plasmid DNA

Transformation of bacteria with resistance genes was carried out as described earlier by Sambrook et al [19] using E. coli k-12 as recipient. Transformants were selected on MHA plates supplemented with trimethoprim- sulfamethoxazole (25µg/mL) and on a nonselective medium. Plates having up to 5 transformants were examined for presence of ESBL and Sul1 genes by PCR with same method and primers as described above. Transformants carrying ESBL and/or Sul1 genes were subsequently tested to the 10 antibiotics as described previously [9].

Ethical statement

The ethical approval was obtained from Delta State University ethics committee with an approval number of EOS20200 and permission to collect samples from hospitals was granted by the medical ethical committee of Delta State hospital management board. Consent from patients or family members was obtained before sample collection.

Results

Resistance profile, Frequency of *Sul* 1 and β-lactamases genes

The isolates showed diversity in resistance patterns resulting in 19, 13 and 18 varied resistance patterns from Warri, Asaba and Agbor locations respectively (Table 2). Phenotypic resistance to one or more of the cephalosporins appears to be a unique feature in the resistance pattern. In at least 95% of the TMSR isolates, amoxicillin-clavulanic acid resistance was observed (Table 2) Resistance to a Fluoroquinolone and trimethoprim-sulfamethoxazole occurred too frequently, appearing almost inseparable. This resistance pair of fluoroquinolone and trimethoprim-sulfamethoxazole was observed in the 3 locations. However, in 36.8%, 30.7% and 16.7% resistance profiles of TMSR isolates in Warri, Asaba and Agbor respectively a fluoroquinolone and trimethoprim-sulfamethoxazole resistance pair were not present (Table 2). Overall, resistance to nitrofurantoin was remarkably lowest in this study.

Among the 92 TMSR isolates, 54 (58.7%) ESBL phenotypes and 27 (29.3%) were identified. ESBL was therefore more prevalent than *Sul* 1. Also, *E. coli* was a more prevalent ESBL producer (75.9%) than *P. aeruginosa* (24.1%) and the detection of Sul 1 and ESBL gene was higher in E. coli than in P. aeruginosa. Additionally, the co-existence of ESBL β-lactamase with *Sul* 1 was frequent and predominantly detected in E. coli isolates (Table 3).

A *blaCTX-M, blaTEM* or *blaSHV* beta-lactamase was identified in 28(30.4%,), 13(14.1%) and 13(14.1%) respectively amongst the ESBL

phenotypes. The *blaCTX-M,* β-lactamase was predominant in *E. coli*, 19(20.7%) (Fig. 1).

Plasmid Analysis and Transformation

The results of plasmid analysis and transformation are presented in Table 5. There were three different sizes of approximately 23.130kbp, 9.416kbp and 1.517kbp encountered and the isolates from Warri harbored two of them simultaneously, irrespective of the sample source. The isolates obtained from Asaba and Agbor harbored single plasmids of any of the sizes encountered (Table 4). Of note is the plasmid size of 23.130kbp carried by isolates in the three locations. Curing of plasmids by SDS indicated that majority of the antimicrobial resistance phenotypes were carried on plasmids and all ESBL positive isolates carried resistance on plasmid. Transformation of Sul 1 gene was observed in 23 (85.2%) isolates, Positive transformants harbored ESBLs genes and /or *Sul* 1 genes of donor isolates. Further examination of the 23 transformed isolates, revealed that in 82.6% case trimethoprim-sulfamethoxazole was transferred and in 60.9% a fluoroquinolone and trimethoprim-sulfamethoxazole was co-transferred.

Table 1. Primer sequence.

Genes	Primer Sequence (5'→3')	Amplicon Size (Bp)	Reference
SHV	F-TCAGCGAAAAACACCTTG	471	
	R-TCCCGCAGATAAATCACC		[16]
TEM	F-CTTCCTGTTTTTGCTCACCCA	717	
	R-TACGATACGGGAGGGCTTAC		[16]
CTX-M 2	F-ATGATGACTCAGAGCATTCG	865	
	R-TGGGTTACGATTTTCGCCGC		[17]
Sul 1	F-CGGATCAGACGTCGTGGATGT	351	[18]
	R-TCGAAGAACCGCACACAATCT CGT		

Table 2. Resistant pattern of TSR E. coli and pseudomonas aeruginosa isolated from hospitalized patients

Bacteria	Resistance Phenotype					
	Warri	No	Asaba	No	Agbor	No
E. coli	A CX CZ CR CF N G C O T	4	A CX CZ CR CF G C O T	2	A CX CZ CR CF N G C O T	1
	A CX CZ CR CF G C O T	6	A CZ CR CF G C O T	4	A CX CZ CR CF G C O T	4
	A CX CZ CF C O T	6	A CX CZ CR CF C O T	1	A CX CZ CF G C O T	1
	A CX CZ CR CF G C T	2	A CF N G O T	1	A CX CZ CR CF O T	1
	A CX CZ CR CF T	3	A CX N G T	1	A CX CZ CR C O T	2
	A CX CZ CF O T	1	A CX CZ CR CF T	1	A CX CZ CR CF T	1
	A CX CZ CF C T	1	CX CZ C O T	3	A CX CZ CR N G T	1
	ACX CR G C O T	1	A CX G O T	2	A CX CZ N G T	1
	A CX CZ T	1	ACX G T	1	A CZ CR G C T	4
	A CX CZ C O T	1			A CZ CR CF T	1
	A CX CZ C T	1			A CZ G C T	1
	A CX CZ N O T	1				
	A CX CR G C T	1				
P. aeruginosa	A CX CZ CR CF G C O T	3	A CX CZ CF G C O T	2	A CX CZ CR CF N G C O T	2
	A CX CZ CR CF G O T	1	A CX CZ CR CF N T	1	A CX CZ CR CF G C O T	3
	A CX CZ CR CF C T	2	A CZ CR CF C O T	1	A CX CZ CR CF C T	1
	A CX CZ CR CF T	1	CX C O T	1	A CX CZ CR CF G O T	1
	A CX CR N T	1			A CX CZ CR G C T	4
	A CZ CR T	1			CX CZ CR CF N C T	1
					CX CZ CR CF C O T	3

Ceftazidime (CZ) Cefotaxime (CF) Cefuroxime (CF), Cefixime (CX), Gentamicin (G), Ciprofloxacin (C), Ofloxacin (O) Trimethoprim-sulfamethoxazole (T), Amoxicillin-clavulanic acid (A) and Nitrofurantoin (N).

Table 3. Prevalence ESBL phenotype and Sul 1 gene in *E. coli* and *P. aeruginosa* isolates obtained from some government hospitals of Delta State.

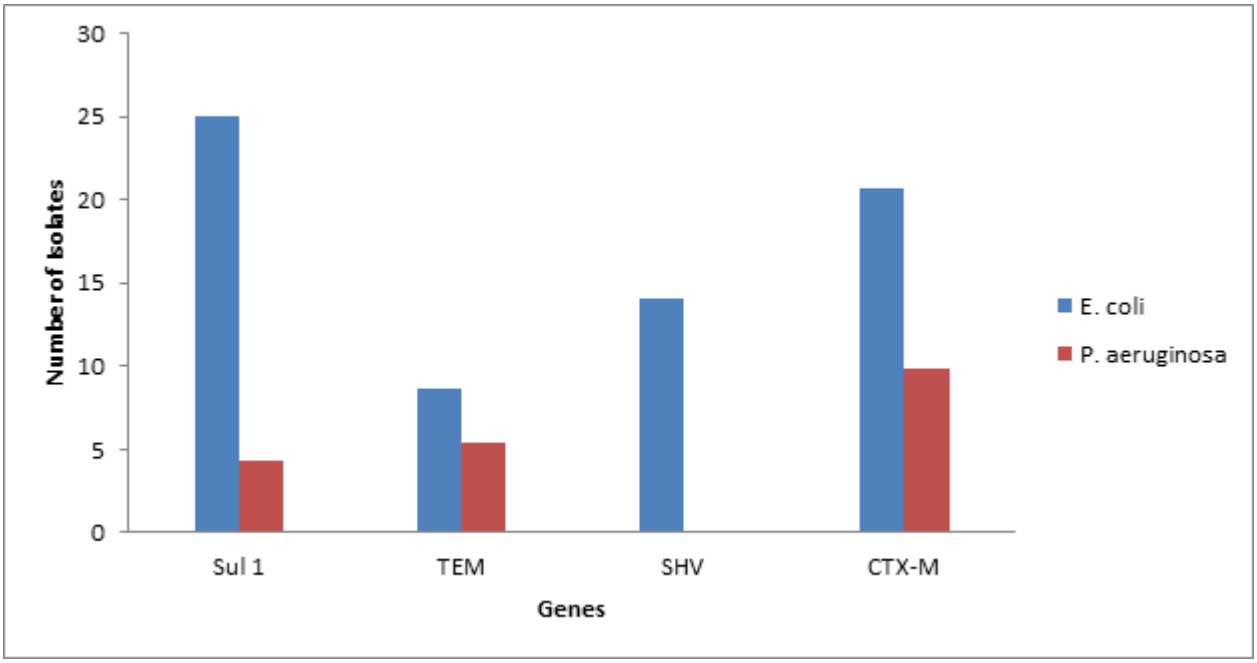
Isolates	Number of isolates *N* =92 [n (%)]	Location	Prevalence [n (%)]		
			ESBL phenotype *N*= 54	*Sul*1 gene *N*= 27	*Sul* 1 + ESBL phenotype *N*= 21
E. coli	29(31.5)	Warri	20(37.0)	12(44.4)	12(57.1)
P. aeruginosa	9(9.8)	Warri	4(7.4)	0(0.0)	0(0.0)
E. coli	18(19.6)	Agbor	12(22.2)	8(29.6)	6(28.6)
P. aeruginosa	15(16.3)	Agbor	5(9.3)	4(14.8)	0(0.0)
E. coli	16(17.4)	Asaba	9(16.7)	3(11.1)	3(14.3)
P. aeruginosa	5(5.4)	Asaba	4(7.4)	0(0.0)	0(0.0)

Table 4. Transformation of plasmid DNA into *E. coli k-12 recipient.*

Source	Bacteria	Resistant phenotype	Antibiotics transferred	Plasmid sizes
Warri	*E. coli*	A CX CZ CR CF N G C O T	A CX CZ CR CF C O T	23.130kbp, 1.517kbp
	E. coli	A CX CZ CR CF N G C O T	CF C O T	23.130kbp, 1.517kbp
	E. coli	A CX CZ CR CF G C O T	A CX CR CF C O T	23.130kbp, 1.517kbp
	E. coli	A CX CZ CR CF G C O T	CX CR CF C	23.130kbp, 1.517kbp
	E. coli	A CX CZ CF C O T	A CZ CF C O T	23.130kbp, 1.517kbp
	E. coli	A CX CZ CR CF G C T	A CR CF C T	23.130kbp, 1.517kbp
	E. coli	A CX CZ CR CF G C O T	A CF G C O T	23.130kbp 1.517kbp
	E. coli	A CX CR G C O T	A C O T	23.130kbp 1.517kbp
Agbor	*E. coli*	A CX CZ CR CF G C O T	-	-
	E. coli	A CX CZ CR CF T	CZ	23.130kbp 1.517kbp
	E. coli	A CX CZ N O T	A T	23.130kbp 1.517kbp
	E. coli	A CX CR G C T	A CX T	23.130kbp 1.517kbp
	E. coli	A CX CZ CR CF N G C O T	A CX CZ CR CF T	23.130kbp
	E. coli	A CX CZ CR CF G C O T	A CX CZ CR C O T	23.130kbp
	E. coli	A CX CZ CF G C O T	A CX CZ C O T	23.130kbp
	E. coli	A CX CZ CR C O T	-	9.416kbp
	E. coli	A CX CZ CR CF O T	A CZ CR CF T	9.416kbp
	E. coli	A CZ CR G C T	A CZ CR T	9.416kbp
Asaba	*E. coli*	A CX CZ CR C O T	A CR C T	9.416kbp
	E. coli	A CX CZ CR CF T	-	23.130kbp
	p. aeruginosa	A CX CZ CR CF N G C O T	A CX CR CF	23.130kbp
	p. aeruginosa	A CX CZ CR CF G C O T	CX CZ	23.130kbp
	P. aeruginosa	A CX CZ CRGC T	CZ CT	9.416kbp
	p. aeruginosa	CX CZ CR CF C O T	CX C O T	9.416kbp
	E. coli	A CX CZ CR CF G C O T	A C T	9.416kbp
	E. coli	CX CZ CO T	-	-
	E, coli	A CZ CR CF G C O T	A CX CZ CR O T	23.130kbp

Ceftazidime (CZ) Cefotaxime (CF) Cefuroxime (CF), Cefixime (CX), Gentamicin (G), Ciprofloxacin (C), Ofloxacin (O) Trimethoprim-sulfamethoxazole (T), Amoxicillin-clavulanic acid (A) and Nitrofurantoin (N).

Figure 1. Percentage distribution of *Sul* 1 and ESBL genes among TSMR isolates



Discussion

The high prevalence of multidrug resistance and ESBL in clinical isolates globally is a worrying issue as it has increased the global disease burden due to ineffectiveness of the available antibiotics. Their presence in clinical isolates of *P. aeruginosa* and *E. coli* with high levels of antimicrobial resistances have been reported in several studies globally [20, 21]. The isolates reported in this present study showed diversity in resistance patterns resulting in 19, 13 and 18 varied resistance patterns from Warri, Asaba, and Agbor locations respectively (Table 2). Phenotypic resistance to one or more of the cephalosporins appears to be a unique feature in the resistance patterns. Resistance to amoxicillin-clavulanic acid was found to be the most prevalent in the trimethoprim-sulfamethoxazole resistant (TSR) (Table 2). The resistant pattern of the TSR isolates indicates co-selection may suggest that they could be linked to the same genetic element. Previous studies [22, 23] observed co-selection of amoxicillin and trimethoprim resistance genes. A predominantly high frequency of resistance to the cephalosporins was also observed.

A similar pattern of resistance to the cephalosporins and amoxicillin-clavulanic acid has been reported in Nigeria [24]. Selective pressure from trimethoprim-sulfamethoxazole may have simultaneously selected for genes mediating resistance to other antibiotics leading to multiple bacterial resistances, causing great difficulties in clinical treatment of infectious diseases and prolonged hospital stay. The clinical and public health implications of the high prevalence of MDR isolates is thus potentially profound. Resistance to fluoroquinolone and trimethoprim-sulfamethoxazole occurred too frequently in this study, appearing almost inseparable. In general, most antimicrobial resistance studies carried out in Nigeria and in many developing countries have reported high prevalence of resistance to cephalosporins, fluoroquinolones and amoxicillin–clavulanic acid. This is a serious therapeutic concern attributed to the extensive and inappropriate use of these antibiotics as first line dose and multiple antibiotic therapies which are prescribed to provide a broader spectrum of activities and synergy in treatment of infections [25]. However, because different bacteria have different resistance mechanisms to different antibiotics co-selection, cross- selection and HGT via resistant plasmids, antibiotic prescription should be guided by resistance testing and local empiric resistance data. Considering the MDR resistance pattern obtained from the study isolates which mirrors the national trend, a more rapid and accurate identification method like using molecular techniques becomes very imperative in order to optimize treatment and reduce resistance evolution arising from inappropriate prescriptions of antibiotics. In

addition, low rate of resistance is achievable through regulatory control of antibiotic usage in humans and veterinary medicine [26; 27].

Our findings provided other insights into the resistance pattern of TSR isolates. The isolates showed low resistance to nitrofurantoin. Trimethoprim-sulfamethoxazole and nitrofurantoin are indicated for use in urinary tract infections [28]. However, in contrast to amoxicillin-clavulanic acid or trimethoprim-sulfamethoxazole, nitrofurantoin resistance genes are not frequently associated with mobile genetic elements carrying multi-drug resistance [29]. Nitrofurantoin use may actually select for bacteria that are susceptible to trimethoprim- sulfamethoxazole [30].

Among the 92 TSR isolates, 54 (58.7%) ESBL phenotypes and 27 (29.3%) Sul1 were identified (Table 3). Sul1 gene and ESBL genotypes were produced in 29.3% and 58.7% of the isolates, respectively (Fig. 1). ESBL was therefore more prevalent than Sul1. Also, E. coli was a more prevalent ESBL producer (75.9%) than P. aeruginosa (24.1%) and the detection of Sul1 and ESBL gene was higher in E. coli than in P. aeruginosa. Additionally, the co-existence of ESBL β-lactamase with Sul1 was frequent and predominantly found in E. coli isolates (Table 3).

Given that the TSR isolates in our study were found to be highly resistant to the fluroquinolones, amoxicillin- clavulanic acid, cephalosporins, and that ESBL producing organisms have been reported to be associated with resistance to these antimicrobial agents, it is therefore not surprising to identify high rates of ESBL producers.

CTX-M was the predominant ESBL identified in this study (Figure 1), this is consistent with global and local reports [9, 31, 32]. ESBLs have been reported to be frequently found in association with *Sul*1 gene [1, 33, 34] as observed in this study. In addition, *Sul*1 gene is part of class 1 integrons, which further enhances the spread of resistance [35, 36]. The results of plasmid analysis and transformation are presented in (**Table 4)**. There were three different sizes of approximately 23.130kbp, 9.416kbp and 1.517kbp encountered and the isolates from Warri harbored two of them simultaneously, irrespective of the sample source. The isolates obtained from Asaba and Agbor harbored single plasmids of any of the sizes encountered. Of note is the plasmid size of 23.130kbp carried by isolates in the three locations. Curing of plasmids by SDS indicated that majority of the antimicrobial resistance phenotypes were carried on plasmids and all ESBL positive isolates harbored resistance on plasmid.

The detection of artificial transformation from *E. coli* and *P. aeruginosa* isolate to E-coli K-12 recipient was successful (Table 4). Transformation of Sul1 gene was observed in 23 (85.2%) isolates, several antibiotics, Sul1 gene as well as ESBL were transferred by transformation. The similar plasmid sizes observed in the different sample sources indicates that these plasmids maybe persistent and thus constituting a reservoir for the dissemination of resistant bacteria carrying plasmids to admitted patients and eventually to the community thereby creating a public health risk.

A rate of 85% (23/27) transformation obtained in this study further confirms antimicrobial resistance gene location on plasmids rather than genome. Many studies [21, 33] have reported the frequency and distribution of *Sul* genes and trimethoprim-sulfamethoxazole resistance in various bacteria, which is a likely indication that these genes are transferred mainly by horizontal routes. That the resistance to trimethoprim-sulfamethoxazole, fluoroquinolones and cephalosporins occurred in unity in the locations and were transferred together suggest that either these resistances were carried on one plasmid facilitating spread or the resistance pattern is endemic in the sampling locations. The possible clonal spread to other parts of the country is underscored. Although transformation was carried out in this study under laboratory conditions, some studies provide evidence demonstrating that natural transformation occur in *E. coli* under divalent metal ions (Ca 2+ and Mg 2+) and biofilms which are prevalent environmental factor in hospital settings [36]. This may present a potential health risk to hospitalized patients and workers as the physical environment in hospitals favors the transmission of resistant bacteria amongst patients and from health care worker or care givers to patients and vice versa via resistance transfer mechanisms.

Conclusion

The resistance to trimethoprim-sulfamethoxazole, fluoroquinolones and the cephalosporins occurred in unity in the locations and were transferred by transformation in the laboratory conditions, the potential for occurrence in clinical or

natural settings cannot be ruled out. Therefore, adequate safety measures to prevent transfer of resistant isolates should be put in place in hospitals so as to stymie the spread of resistance.

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Author contributions

Conception: OSE and BOE, BCI

Design: . OSE, BOE, UBO and OO

Interpretation: BOE, UBO and OO, BCI, LCO

Writing the manuscript: OSE, BCI, LCO

Conflict of interest.

The authors declare that they have no conflict of interest.

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