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ORIGINAL ARTICLE

High Prevalence of Phenotypic Resistance to Colistin, Tigecycline and Netilmicin in a Region with no History of Colistin Administration in Nigeria

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SUMMARY

Background: **Colistin is currently used as a last line antibiotic for the treatment of extensively drug-resistant Gram-negative bacteria. The widespread dissemination of ESBL-producing and carbapenem-resistant Gram-negative bacteria necessitated the re-introduction of colistin which was initially abandoned as a result of high toxicities. This study aimed at determining the epidemiology of *Enterobacteriaceae* isolates that are resistant to colistin, tigecycline, and netilmicin and the risk factors that may contribute to the resistance observed within Southeastern Nigeria.**

Methods: **A total of 400 participants who came to the hospitals for various reasons and suspected to have bacterial infections were enrolled in this study. Phenotypic resistance to colistin was detected using broth microdilution technique on cation - adjusted Mueller-Hinton broth while resistance to colistin and netilmicin was detected using the Kirby-Bauer disk diffusion method using tigecycline disk (10 µg) and netilmicin disk (30 µg). Risk factors were determined by the administration of questionnaires to the participants.**

Results: **Our study found an overall high prevalence of colistin - resistant *Enterobacteriaceae* (45.8%) and a high prevalence for colistin and tigecycline (22.2%) and colistin and netilmicin (31.7%) resistant isolates. Among the risk factors that contribute to colistin, non-completion of antibiotics was found to be more significant (p = 0.039 and an odds ratio of 0.324 - 0.972 at 95% confidence interval).**

Conclusions: **Our finding is significant in that colistin resistance was determined to be present among populations that have not been exposed to colistin therapy.**

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INTRODUCTION

Since the first emergence of antibiotic resistance, there has been increased development and spread of resistance genes to other organisms. Most often, antibiotic resistance arises from either mutation or by the acquisition of antibiotic genes through horizontal gene transfer. However, antimicrobial resistance is frequently mediated by the acquisition of genes which are carried on mobile genetic elements such as plasmids, insertion sequences, transposons, and integrons. As such, they can

easily be transferred among bacteria of different species and genera, leading to the progressive diminution of antimicrobial activity in both the hospital and in the community and culminating in the emergence of multidrug resistant and extensively drug resistant organisms [1,2]. Colistin is currently used as a last line antibiotic for the treatment of extensively drug-resistant Gram-negative bacteria. It is highly effective for the treatment of most Gram-negative infections. This is why resistance to colistin is a serious problem because there is no other alternative antibiotic for the treatment of these infections [2].

Colistin was isolated from the bacterium *Paenibacillus polymyxa* subspecies *colistinus* in 1947 [3]. It is a polycationic antibiotic with significant activity against Gram-negative bacteria such as *Enterobacteriaceae*. The main site of action for colistin is the outer cell membrane of Gram-negative bacteria. When colistin binds to lipopolysaccharides in the outer membrane, electrostatic interaction occurs which competitively displaces divalent cations Ca^{2+} and Mg^{2+} from the phosphate groups of membrane lipids leading to increased permeability of the outer membrane and leakage of intracellular contents leading to cell death [4,5].

The widespread dissemination of ESBL-producing and carbapenem-resistant Gram-negative bacteria necessitated the re-introduction of colistin which was initially abandoned as a result of high toxicities. However, this caused excessive dependence on colistin and mounted pressure which culminated in the emergence of resistance to colistin. Resistance to colistin occurs either by chromosomal mutations that mediate the alteration of the lipid A moiety of the lipopolysaccharide or by the acquisition of the plasmid-mediated *MCR* gene [6-9]. Resistance in Gram-negative organisms has increased rapidly and is associated with increased morbidity, mortality, and increased costs of treatment. Colistin is used to treat *Enterobacteriaceae* infections that have developed resistance to the cephalosporins and to the carbapenems. The challenge is that strains that are resistant to carbapenems simultaneously manifest resistance to other antibiotics such as fluoroquinolones, aminoglycosides, tetracycline and trimethoprim-sulfonamides; therefore, the only available option for their treatment is colistin. The problem is therefore complicated when these organisms develop resistance to colistin. Resistance to colistin can emerge through multiple mechanisms such as loss of polysaccharides, modification of lipopolysaccharides, and increased capsule production [3,10].

Tigecycline is a derivative of minocycline and is in the class of glycylcyclines. It is bacteriostatic and has a broad spectrum of activity including aerobic and anaerobic Gram-positive and Gram-negative organisms. Tigecycline has been shown to be effective against multidrug-resistant *Enterobacteriaceae* including those that are resistant to other tetracyclines [11]. Tigecycline was shown by some studies to be effective against infections that were resistant to the cephalosporins and to imipenem - a carbapenem [12]. Therefore, it is used with colistin for the treatment of extensively drug-resistant infections. It acts by binding irreversibly to the 16S rRNA and S12 protein of the bacterial 30s ribosomal subunit and, as such, interferes with and inhibits protein synthesis. It also induces misreading of mRNA template causing translational frameshift leading to the premature termination of translation. Colistin and tigecycline are among the very few agents that show excellent activities against carbapenem-resistant *Enterobacteriacae* but due to their widespread dependence and use, these last resort antimicrobial agents have been challenged by antimicrobial resistance [13].

The best approach towards combating antimicrobial resistance is to suppress further emergence of resistant strains because the pace of developing new effective antibiotics is slow [13]. One strategy towards achieving this is to determine the epidemiology of multidrug-resistant, extensively drug-resistant, and pandrug resistant isolates and then design the best protocol of antibiotics to be used in the treatment of infections caused by these strains or a combination of antibiotics that have synergistic mechanisms of action. Zhao and Drlica [14] proposed the simultaneous administration of two antibiotics with different modes of action and without occurrence of cross resistance.

Netilmicin is a semi-synthetic aminoglycoside produced by the fermentation of the actinomycete *Micromonospora inyioensis.* It is effective against infections caused by aerobic Gram-negative organisms. Resistance to netilmicin occurs through modification of the target 16S rRNA and this could be mediated by the enzymes 16S rRNA methyltransferases and 16S methylases. The genes coding for these enzymes can be carried on plasmids, transposons or class 1 integrons and, as such, they can easily be spread to other organisms by various mechanisms of horizontal gene transfer [15].

The burden of antimicrobial resistance in developing countries cannot be overemphasized. Founou et al. [16] posited that antimicrobial resistance is responsible for high morbidity, high mortality, and increased economic costs, and patients with non-communicable disease comorbidities were identified as high risk populations. Epidemiological studies on colistin, netilmicin, and tigecycline resistant *Enterobacteriaceae* have become necessary to provide data required for the complete understanding of the global distribution of extensively resistant and pandrug resistant isolates [15,17]. This will serve as a guide in the rational design of effective therapeutic regimens. Such information could be useful for directing hospital resources to prevent the nosocomial spread of these resistant strains.

Accurate identification of colistin-resistant bacteria is of critical importance in the efforts to limit further dissemination of plasmid-mediated genes that confer resistance to colistin. Phenotypic detection of colistin resistance is at present the mainstay of antimicrobial resistance surveillance and is necessary to determine the occurrence of colistin resistance within the global antimi-

crobial resistance surveillance system [18].
This study aimed at determining the epidemiology of *Enterobacteriaceae* isolates that are resistant to colistin, tigecycline, and netilmicin and to also determine the risk factors that may result to the extensively drug-resistant phenomenon in *Enterobacteriaceae* isolates.

MATERIALS AND METHODS

Study area

South-eastern Nigeria lies within the coordinates $5^{o}25'N8^{o}05'E$ and is made up of five States; Abia, Anambra, Ebonyi, Enugu, and Imo. It has an area of approximately 76,000 square kilometers. According to the 2006 census, the population of the region was approximately 16,395,555. The region has three types of vegetation; mangrove swamps and tidal waterways dominate the coastal areas; tropical rainforests dominate the regions further north of the swamps while guinea savannah dominates the northern most parts of the region.

Study design and Sampling technique

This was a cross-sectional study designed and carried out across five tertiary hospitals in South-eastern Nigeria. Among eleven tertiary hospitals that are located within the region, five of them were selected using simple random sampling technique.

Study population

The participants were patients who presented with clinical manifestations that suggested the presence of infection(s) with any of the *Enterobacteriaceae* based on the provisional diagnosis and who had laboratory requests for microscopy, culture, and sensitivity. Specimens were collected from the participants as requested in their laboratory forms and the isolates were identified to species level. Participants from whom *Enterobacteriaceae* were not isolated from their specimens were removed from the study. A total of 400 *Enterobacteriaceae* isolates were obtained from the participants.

Ethical consideration

Ethical approval was obtained from the ministries of health of Abia, Ebonyi, Enugu, Imo and Anambra States. Informed consent was obtained from the participants or from their parents/guardians (for those less than 18 years old). The participants were assured that their identities would not be linked to any data. The study was conducted following strict adherence to international, national, and institutional ethical guidelines and in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Sample size

The sample size was calculated using the formula; $n = Z^2PQ/d^2$ where; n = minimum sample size, Z = 1.96 (Standard deviation of a normal distribution taken at 95% confidence interval which corresponds to 1.96), d = 0.05 (degree of accuracy set at 0.05 for 95% confidence interval, and p = 50% (expected prevalence from available literature; however, no previous epidemiological study of this nature has been conducted within the study area; hence, a prevalence of 50% was assumed), and Q = 1 - p. Therefore, $n = (1.96)^2 \times 0.5 (1 - 0.5)/(0.05)^2$ gives an estimated sample size of 384, which was increased to 400. To determine the number of samples that would be collected from each center, the probability proportion by size was calculated. To determine this, the average number of patients received in the medical microbiology laboratory from each center per month was obtained. Total number of samples: 900 + 1,200 + 1,350 + 600 + 600 = 4,650.
The formula used to calculate the sample size to be collected from each center was
a/b x n
where; a = average total samples received by each centre per month, b = total number of samples, n = sample size (400). Hence, the sample size obtained from each center is presented in Table 1.

Inclusion/Exclusion criteria

Patients who gave informed consent and in whom *Enterobacteriaceae* organisms were isolated from their specimens were included in this study. Patients who gave consent but in whom *Enterobacteriaceae* was not isolated from their specimens were excluded from the study. Also, based on the prescriptions on the patients' folders and on verbal interview, patients that were on combined antibiotic therapy were excluded from the study.

Specimen collection and identification

Specimens were collected from the participants based on the requests on their laboratory request forms. *Enterobacteriaceae* were isolated from various specimens such as urine, sputum, cerebrospinal fluids, stool, blood, semen, wound, high vaginal, ear, throat, urethral, and eye swabs. The identification of the isolates was performed using standard microbiological methods described by Cheesbrough [19] and Forbes et al. [20], which include Gram reaction and conventional biochemical tests such as indole, methyl red, Voges-Proskauer, citrate utilization, oxidase, urease, triple sugar iron, and sugar fermentation reactions.

Questionnaire

Questionnaires were administered to the patients when they visited the clinics. Each questionnaire was administered to the respondents after proper instructions had been given and understood. They were retrieved after completion and documented.

Detection of colistin resistance

Phenotypic resistance to colistin was detected using methods described by EUCAST guidelines [21]. Broth microdilution method using cation-adjusted Mueller-Hinton broth (Oxoid, Batch number 2372007) was used.

Three microtiter wells were used for each isolate, each of the tubes having a concentration of 2 µg/mL, 4 µg/mL, and 6 µg/mL of colistin sulphate respectively. A suspension of the bacteria was made in sterile normal saline to match 0.5 McFarland turbidity equivalent standard. Subsequently, an inoculum size of 0.1 mL was used to inoculate the media and then incubated at 37°C for 18 hours. MIC was determined as the lowest concentration which showed inhibition of growth of the isolates by the absence of turbidity. Results were interpreted based on EUCAST guidelines [21].

Detection of resistance to Tigecycline and Netilmicin

Resistance to Tigecycline (10 µg) and Netilmicin (30 µg) (Oxoid, Batch Numbers 2426983 and 2261751, respectively) were detected using Kirby-Bauer disk diffusion method. The results were interpreted based on guidelines of EUCAST version 8.1 [21].

Data analysis

Data were analyzed with the aid of Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive analysis, frequency tables and percentages were used for the univariate analysis while chi-square test was used for the bivariate analysis. Fisher's exact test and logistic regression was used for the multivariate analysis. p-value < 0.05 was considered significant in all analyses.

RESULTS

A total of 400 persons participated in this study, they comprised of 208 (52.0%) females and 192 (48.0%) males. The gender ratio was approximately 1:1, the age groups of the participants ranged from 3 years to above 70 years. However, the age groups with the highest participants were in the range of 20 - 29 years (31.0%) while the least were those in the 50 - 59 years age group (7.0%) (Table 2).

The frequency distribution of the isolates among the participants is presented in Table 3. *E. coli* had the highest prevalence of 30.8% followed by *Klebsiella pneumonia* (19.3%) and *Citrobacter freundii*. *Yersinia enterocolytica* had the least prevalence of 0.3%. The distribution of these isolates among the specimens from where they were obtained is presented in Table 4. The frequency distribution of the specimens obtained for this study is presented in as Figure 1. Urine was the most common specimen (34.3%). This was followed by wound swab (14.3%) and stool (12.5%). Eye swab was the least common among the specimens (0.3%) obtained from the participants.

A total of 183 (45.8%) of the isolates were resistant to colistin (with MIC above 2 µg/mL). Among these, *Shigella dysenteriae* (47.1%) showed the highest resistance. This was followed by *Enterobacter cloacae* (38.9%), *Proteus vulgaris* (38.5%), and *Klebsiella oxytoca* (35.7%). However, when the results of organisms that are intrinsically resistant to colistin (*Morganella morganii, Serratia marcescens, Proteus mirabilis,* and *Proteus vulgaris*) are removed, the actual prevalence of colistin resistance observed for organisms that should be susceptible to colistin was 32.0% (102 of 319) (Table 4). There was a significant relationship in the distribution of colistin resistance among the isolates (p = 0.000).

The prevalence of isolates that were resistant to tigecycline was 39.0% (Table 5) and the relationship between the isolates and resistance to tigecycline was non-significant (p = 0.680). Also, the overall resistance to netilmicin was 67.2% (Table 6) and the relationship between the isolates and resistance to netilmicin was non-significant (p = 0.100).

When the susceptibility pattern of the isolates that were simultaneously resistant to both colistin and tigecycline were observed, a total of 89 (22.2%) were resistant to both colistin and tigecycline. Among these, the highest resistance was observed in *Shigella dysenteriae* (35.3%), followed by *Proteus vulgaris* (30.8%) and *Enterobacter cloacae* (22.2%). The least resistant to both was *Citrobacter freundii* (2.4%). There was a significant relationship between the distribution of the isolates and resistance to both colistin and tigecycline as presented in Table 7.

As shown in Table 8, a total of 127 (31.7%) isolates were resistant to both netilmicin and colistin. Among these, the highest resistance was observed in *Shigella dysenteriae* (47.1%). This was followed by *Enterobacter cloacae* (33.3%) and *Proteus vulgaris* (30.8%), and there was no significant relationship between the isolate distribution and resistance to both colistin and netilmicin (p = 0.205).

The highest colistin-resistant isolates were from blood cultures (41.2%), followed by sputum (37.5%), while urethral swab was the least resistant specimen from which colistin-resistant *Enterobacteriaceae* was isolated. There was no significant relationship between the specimen distribution and colistin resistance (p = 0.627).

When the distribution of colistin resistant isolates was compared among the centers studied, Enugu state had the highest prevalence (32.8%) while Anambra state had the least prevalence (17.3%). There was no significant relationship between the states and the presence of colistin-resistant isolates (p = 0.095) (Table 9).

The frequency distribution of the risk factors showed that most of the participants 255 (63.8%) were from urban areas, while 145 (36.3%) were from rural areas. The majority of the participants 339 (84.3%) were not on current medications, while only 61 (15.3%) of them were on current medication. Out of the 61 participants that were on medication, 44 (72.1%) of them were on antibiotic, while the remaining 17 (27.9%) were on non-antibiotic medication. Less than half of the participants 173 (43.3%) took drugs without prescription by a doctor and 170 (42.5%) of them always completed their dosage as prescribed. Most of the participants 273 (68.3%)

Table 1. Sample size calculation from the probability proportion by size.

S/N	Centre	(a)	Sample size (n)
1	Federal Medical Centre Umuahia, Abia State	900	77
2	Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State	1,200	103
3	Enugu State University Teaching Hospital, Parklane, Enugu State	1,350	116
4	Imo State University Teaching Hospital, Imo State	600	52
5	Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Anambra State	600	52
	Total (b)	4,650	400

Table 2. Frequency distribution of the demographic variables, n = 400.

Demographic variables	Frequency	Percentage
State		
Abia	77	19.3%
Ebonyi	103	25.8%
Enugu	116	29.0%
Imo	52	13.0%
Anambra	52	13.0%
Age group (years)		
< 20	71	17.8%
20 - 29	124	31.0%
30 - 39	46	11.5%
40 - 49	35	8.8%
50 - 59	28	7.0%
60 - 69	66	16.5%
70 & above	30	7.5%
Gender		
Male	192	48.0%
Female	208	52.0%

Table 3. Frequency distribution of the isolates, n = 400.

Isolate	Frequency	Percentage
Escherichia coli	123	30.8%
Klebsiella pneumoniae	77	19.3%
Citrobacter freundii	41	10.3%
Klebsiella oxytoca	28	7.0%
Morganella morganii	27	6.8%
Serratia marcescens	22	5.5%
Proteus mirabilis	19	4.8%
Enterobacter cloacae	18	4.5%
Shigella dysenteriae	17	4.3%
Salmonella enterica	14	3.5%
Proteus vulgaris	13	3.3%
Yersina enterocolytica	1	0.3%

Table 4. Relationship of the isolate distribution and colistin resistance, n = 400.

Isolate	Colistin resistant			χ^2	p-value
	S	R	Total		
Escherichia coli	88 (71.5%)	35 (28.5%)	123 (100%)	135.86	0.000
Klebsiella pneumoniae	57 (74.0%)	20 (26.0%)	77 (100%)		
Citrobacter freundii	22 (78.6%)	19 (21.4%)	41 (100%)		
Klebsiella oxytoca	18 (64.3%)	10 (35.7%)	28 (100%)		
Morganella morganii	0 (0.00%)	27 (100%)	27 (100%)		
Serratia marcescens	0 (0.00%)	22 (100%)	22 (100%)		
Proteus mirabilis	0 (84.2%)	19 (100%)	19 (100%)		
Enterobacter cloacae	11 (61.1%)	7 (38.9%)	18 (100%)		
Shigella dysenteriae	9 (52.9%)	8 (47.1%)	17 (100%)		
Salmonella enterica	11 (78.6%)	3 (21.4%)	14 (100%)		
Proteus vulgaris	0 (61.5%)	13 (100%)	13 (100%)		
Yersinia enterocolytica	1 (100%)	0 (0.0%)	1 (100%)		
Total	217 (54.2%)	183 (45.8%)	400(100%)		

Table 5. Relationship of the isolate distribution and tigecycline resistance, n = 400.

Isolate	Tigecycline resistance			χ^2	p-value
	S	R	Total		
Escherichia coli	73 (59.3%)	50 (40.7%)	123 (100%)	8.372	0.680
Klebsiella pneumoniae	49 (63.6%)	28 (36.4%)	77 (100%)		
Citrobacter freundii	30 (73.2%)	11 (26.8%)	41 (100%)		
Klebsiella oxytoca	18 (64.3%)	10 (35.7%)	28 (100%)		
Morganella morganii	18 (66.7%)	9 (33.3%)	27 (100%)		
Serratia marcescens	11 (50.0%)	11 (50.0%)	22 (100%)		
Proteus mirabilis	12 (63.2%)	7 (36.8%)	19 (100%)		
Enterobacter cloacae	9 (50.0%)	9 (50.0%)	18 (100%)		
Shigella dysenteriae	10 (58.8%)	7 (41.2%)	17 (100%)		
Salmonella enterica	6 (42.9%)	8 (57.1%)	14 (100%)		
Proteus vulgaris	7 (53.8%)	6 (46.2%)	13 (100%)		
Yersinia enterocolytica	1 (100%)	0 (0.0%)	1 (100%)		
Total	244 (61.0%)	156 (39.0%)	400 (100%)		

bought their drugs from pharmacy, while 77 (19.3%) of them bought theirs from a patent medicine store and 50 (12.5%) bought theirs from the hospital. Very few of the participants 58 (14.5%) visit hospital frequently and only 37 (9.3%) of them had been hospitalized in the past six months. Among these 37 participants that had been hospitalized in the past six months, about half of them 20 (54.1%) spent 1 month in the hospital, while 8 (21.6%) of them spent more than 3 months but only 2 (5.4%) of them spent less than 1 month in the hospital. Very few of the participants 51 (12.8%) had sick relatives living in their house (Table 11).

The logistic regression showed that there is a significant relationship between dosage completion as prescribed and colistin resistance ($p < 0.05$). This implies that those that do not always complete their dosage as prescribed were two times less likely to be resistant to colistin than those that always complete their dosage as prescribed (OR = 0.561, 95% CI for OR = 0.324 - 0.972) (Table 12).

Table 6. Relationship of the isolate distribution and netilmicin resistance, n = 400.

Isolate	Netilmicin resistance			χ^2	p-value
	S	R	Total		
Escherichia coli	36 (29.3%)	87 (70.7%)	123 (100%)	17.285	0.100
Klebsiella pneumoniae	21 (27.3%)	56 (72.7%)	77 (100%)		
Citrobacter freundii	22 (53.7%)	19 (46.3%)	41 (100%)		
Klebsiella oxytoca	11 (39.3%)	17 (60.7%)	28 (100%)		
Morganella morganii	8 (29.6%)	19 (70.4%)	27 (100%)		
Serratia marcescens	7 (31.8%)	15 (68.2%)	22 (100%)		
Proteus mirabilis	8 (42.1%)	11 (57.9%)	19 (100%)		
Enterobacter cloacae	3 (16.7%)	15 (83.3%)	18 (100%)		
Shigella dysenteriae	4 (23.5%)	13 (76.5%)	17 (100%)		
Salmonella enterica	4 (28.6%)	10 (71.4%)	14 (100%)		
Proteus vulgaris	7 (53.8%)	6 (46.2%)	13 (100%)		
Yersinia enterocolytica	0 (100%)	1 (100%)	1 (100%)		
Total	131 (32.8%)	269 (67.2%)	400 (100%)		

Table 7. Relationship of the isolate distribution with tigecycline and colistin resistance, n = 400.

Isolate	Tigecycline and Colistin resistance			χ^2	p-value
	S	R	Total		
Escherichia coli	99 (80.5%)	24 (19.5%)	123 (100%)	51.037	0.000
Klebsiella pneumoniae	63 (81.8%)	14 (18.2%)	77 (100%)		
Citrobacter freundii	40 (97.6%)	1 (2.4%)	41 (100%)		
Klebsiella oxytoca	24 (85.7%)	4 (14.3%)	28 (100%)		
Morganella morganii	18 (66.7%)	9 (33.3%)	27 (100%)		
Serratia marcescens	11 (50.0%)	11 (50.0%)	22 (100%)		
Proteus mirabilis	12 (63.2%)	7 (36.8%)	19 (100%)		
Enterobacter cloacae	14 (77.8%)	4 (22.2%)	18 (100%)		
Shigella dysenteriae	11 (64.7%)	6 (35.3%)	17 (100%)		
Salmonella enterica	11 (78.6%)	3 (21.4%)	14 (100%)		
Proteus vulgaris	7 (53.9%)	6 (46.1%)	13 (100%)		
Yersinia enterocolytica	1 (100%)	0 (0.0%)	1 (100%)		
Total	311 (77.8%)	89 (22.2%)	400 (100%)		

DISCUSSION

Our study found an overall high prevalence of colistin-resistant *Enterobacteriaceae* (45.8%) among populations that have not been exposed to colistin as a therapeutic regimen. However, organisms like *Morganella morganii, Serratia marcescens, Proteus mirabilis,* and *Proteus vulgaris* showed complete resistance to colistin. This observation was because these organisms are intrinsically resistant to colistin [18]. Hence, when their results are removed, the actual resistance to colistin was 32.0%. Our finding of high colistin resistance is comparable to the reports of Jafari et al. [22] who reported a prevalence of 50% of colistin resistant isolates in Iran. When the distribution of colistin-resistant isolates was compared among the states of the southeast region studied, Ebonyi state had the highest prevalence (33.0%) closely followed by Enugu State (32.8%) while the state with the least prevalence was Anambra state (15.4%). This finding calls for a review and possible overhaul of

Table 8. Relationship of the isolate distribution with netilmicin and colistin resistance, n = 400.

Isolate	Netilmicin and Colistin resistant			χ^2	p-value
	S	R	Total		
Escherichia coli	91 (74.0%)	32 (26.0%)	123 (100%)	14.523	0.205
Klebsiella pneumoniae	58 (75.3%)	19 (24.7%)	77 (100%)		
Citrobacter freundii	38 (92.7%)	3 (7.3%)	41 (100%)		
Klebsiella oxytoca	23 (82.1%)	5 (17.9%)	28 (100%)		
Morganella morganii	8 (29.6%)	19 (70.4%)	27 (100%)		
Serratia marcescens	7 (31.8%)	15 (68.2%)	22 (100%)		
Proteus mirabilis	8 (42.1%)	11 (57.9%)	19 (100%)		
Enterobacter cloacae	12 (66.7%)	6 (33.3%)	18 (100%)		
Shigella dysenteriae	9 (52.9%)	8 (47.1%)	17 (100%)		
Salmonella enterica	11 (78.6%)	3 (21.4%)	14 (100%)		
Proteus vulgaris	7 (53.8%)	6 (46.2%)	13 (100%)		
Yersinia enterocolytica	1 (100%)	0 (0.0%)	1 (100%)		
Total	273 (68.3%)	127 (31.7%)	400 (100%)		

Table 9. Relationship between the specimen distribution and colistin resistance, n = 400.

Specimen	Colistin resistance			χ^2	p-value
	S	R	Total		
Urine	98 (71.5%)	39 (28.5%)	137 (34.3%)	9.879	0.627
Stool	38 (76.0%)	12 (24.0%)	50 (12.5%)		
W/S	41 (82.0%)	9 (18.0%)	50 (12.5%)		
Blood	20 (58.8%)	14 (41.2%)	34 (8.5%)		
HVS	26 (81.2%)	6 (18.8%)	32 (8.0%)		
ECS	22 (75.9%)	7 (24.1%)	29 (7.3%)		
Semen	14 (82.4%)	3 (17.6%)	17 (4.3%)		
Sputum	9 (64.3%)	5 (37.5%)	14 (3.5%)		
Ear swab	8 (72.7%)	3 (27.3%)	11 (2.8%)		
Throat swab	8 (80.0%)	2 (20.0%)	10 (2.5%)		
Urethral swab	7 (87.5%)	1 (12.5%)	8 (2.0%)		
Wound swab	5 (71.4%)	2 (28.6%)	7 (1.8%)		
Eye swab	1 (100%)	0 (0.0%)	1 (0.3%)		
Total	283 (70.8%)	117 (29.2%)	400 (100%)		

Table 10. Relationship between the state distributions and colistin resistance, n = 400.

State	Colistin resistance			χ^2	p-value
	S	R	Total		
Abia	56 (72.7%)	21 (27.3%)	77 (19.3%)	7.919	0.095
Ebonyi	69 (67.0%)	34 (33.0%)	103 (25.8%)		
Enugu	78 (67.2%)	38 (32.8%)	116 (29.0%)		
Imo	41 (78.8%)	11 (21.2%)	52 (13.0%)		
Anambra	44 (84.6%)	8 (15.4%)	52 (13.0%)		
Total	288 (72.0%)	112 (28.0%)	400 (100%)		

Table 11. Frequency distributions of the potential risk factors, n = 400.

Risk factors	Frequency	Percentage
Residence		
Urban	**255**	**63.8%**
Rural	**145**	**36.3%**
Current medications		
Yes	**61**	**15.3%**
No	**145**	**84.7%**
Is the medication an antibiotic?		
Yes	**44**	**72.1%**
No	**17**	**27.9%**
Total	**61**	**100%**
Do you take drugs without prescription by a doctor?		
Yes	**173**	**43.3%**
No	**227**	**56.7%**
Do you always complete your dosage as prescribed?		
Yes	**170**	**42.5%**
No	**56**	**14.0%**
Not always	**174**	**43.5%**
Where do you buy your drugs?		
Hospital	**50**	**12.5%**
Pharmacy	**273**	**68.3%**
Patent Medicine Store	**77**	**19.3%**
Do you visit the hospital frequently?		
Yes	**58**	**14.5%**
No	**342**	**85.5%**
Have you been hospitalized for the past six months?		
Yes	**37**	**9.3%**
No	**363**	**90.8%**
If yes, for how many months?		
Less than 1month	**2**	**5.4%**
1 month	**20**	**54.1%**
2 months	**6**	**16.2%**
3 months	**1**	**2.7%**
More than 3 months	**8**	**21.6%**
Total	**37**	**100%**
Do you have a sick relative living in your house?		
Yes	**51**	**12.8%**
No	**349**	**87.3%**

the protocol for administration of antibiotics in hospitals. This is because colistin resistance is reported to have arisen and spread as a result of antibiotic overuse in hospitals [6]. However, comparable to our findings, Hu et al. [23] reported the presence of colistin resistance in clinical isolates even without colistin treatment.

Colistin resistance may arise and spread rapidly because the mechanisms involve both chromosomal mutation and acquisition of plasmid-mediated genes by horizontal gene transfer. While several studies in different countries have reported the presence of colistin resistance among humans and in animals [9], there is a pau-

Table 12. Logistic regression of the relationship between distributions of the risk factors and colistin resistance.

Risk factors	Coefficient	p-value	OR	95% CI for OR
Residence				
Urban (Ref.)				
Rural	**-0.038**	**0.879**	**0.963**	**0.590 - 1.570**
Current medications				
Yes (Ref.)				
No	**-0.182**	**0.575**	**0.833**	**0.441 - 1.576**
Do you take drugs without prescription by a doctor?				
Yes (Ref.)				
No	**0.285**	**0.261**	**1.330**	**0.809 - 2.185**
Do you always complete your dosage as prescribed?				
Yes (Ref.)				
No	**0.099**	**0.777**	**1.105**	**0.555 - 2.197**
Not always	**-0.578**	**0.039**	**0.561**	**0.324 - 0.972**
Where do you buy your drugs?				
Hospital (Ref.)				
Pharmacy	**0.312**	**0.417**	**1.366**	**0.643 - 2.901**
Patent Medicine Store	**0.376**	**0.421**	**1.456**	**0.583 - 3.640**
Do you visit the hospital frequently?				
Yes (Ref.)				
No	**0.001**	**0.997**	**1.001**	**0.504 - 1.991**
Have you been hospitalized for the past six months?				
Yes (Ref.)				
No	**0.393**	**0.398**	**1.481**	**0.596 - 3.683**
Do you have a sick relative living in your house?				
Yes (Ref.)				
No	**0.053**	**0.884**	**1.054**	**0.518 - 2.145**

OR - Odds Ratio, CI - Confidence Interval.

city of data in Nigeria and no study has been conducted in the Southeastern region; hence, our findings provide the first report on the epidemiology of colistin resistant *Enterobacteriaceae* in Southeastern Nigeria. It therefore calls for more studies to delineate the pattern of colistin resistance within the region.

A prevalence of 0.96% of colistin-resistant bacteria was identified from poultry samples, and there was a high level of colistin resistance in the identified isolates in Iran [24]. Colistin is widely used in veterinary medicine to prevent and treat infections caused by *Enterobacteriaceae* and other Gram-negative bacteria and for growth promotion. It is mostly used in food-producing animals such as hens, cattle, and goats [25]. When humans consume such products, they also take in the colistin albeit at a much lower dose and this creates a selective pressure for the spread of colistin resistance in humans. Furthermore, it was initially thought that resistance to colistin is only chromosomally mediated and is not transferrable. But in 2015, a plasmid-mediated gene that confers resistance to colistin, *MCR-1* was first reported in *E. coli* isolates from food animals and their meat and in *E. coli* and *K. pneumonia* isolates obtained from human patients in China in 2014 [26], thereby signaling the mechanism of extensive spread via horizontal gene transfer.

Colistin resistance was observed more in blood culture specimens (41.2%) than in isolates obtained from other specimens. When systemic infections manifest resistance to the last resort antibiotic it limits the chances for survival. This finding therefore signals the urgent need for measures to be instituted towards absolute eradication of antimicrobial resistance.

Among the different risk factors that were compared with the presence of colistin-resistance in this study, non-completion of dosage was most significant and cor-

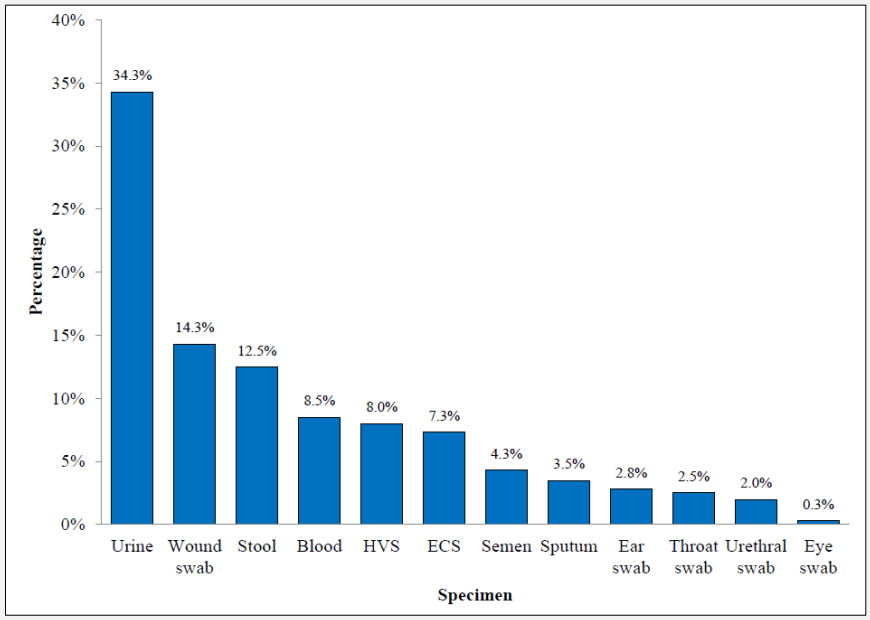

Figure 1. Frequency distribution and percentages of the specimens.

related with the presence of colistin resistance. When the prescribed dosage is not completed, the organisms are exposed to low concentrations of the antibiotics and this mounts sufficient selective pressure that culminates in the development and emergence of antimicrobial resistance. This finding is comparable to that of Büchler et al. [27] who found prior exposure to carbapenems as the only risk factor for colonization or infection with colistin-resistant *E. coli* or *K. pneumonia*.

When the results of the resistance to tigecycline and netilmicin are compared, tigecycline showed a lower prevalence of resistance to the organisms (39.0%), and it was significant ($p = 0.000$) while netilmicin resistance was 67.2% and it was non-significant ($p = 0.205$). While both of them are potentially good options for combination therapy with colistin, tigecycline is a better option than netilmicin.

The finding of simultaneous resistance to colistin and tigecycline as found in this study (22.2%) is in agreement with the reports of previous studies [28,29]. Colistin appears to be a poor monotherapeutic option [30], hence the need to use combination therapy with colistin, using an antibiotic that exerts synergistic effect with colistin. Tigecycline was found to be highly effective against carbapenem-resistant *Klebsiella pneumonia* [12, 22]. This study found a lower prevalence for colistin and tigecycline (22.2%) than for colistin alone (32.0%). This finding suggests that combination therapy should guarantee adequate dosage administration because low drug concentrations can easily select resistant subpopulations of colistin and tigecycline and lead to rapid development of resistance.

This study observed reduced prevalence of resistance in organisms that were resistant to both colistin and netilmicin (31.7%) and to colistin and tigecycline (22.2%). This suggests that tigecycline is a better antibiotic to be used in combination with colistin than netilmicin and should be considered when decision is being taken on drugs to be administered in extensively drug resistant infections. There was no data on tigecycline and netilmicin combinations to compare with the results of this study.

Multidrug resistant Gram-negative organisms are increasing and factors such as infection control measures, lack of antimicrobial control such as the presence of active antimicrobial stewardship teams in the hospitals as well as international travel favor their continuous dissemination [31]. Ouedraogo et al. [32] posited that to limit the dissemination of genes responsible for antimicrobial resistance, public health efforts should be directed towards mass education of both the population and healthcare professionals as well as surveillance and promotion of correct and restricted antibiotic use. The findings of this study are in agreement with this view.

CONCLUSION

Our study found a high prevalence of colistin resistant *Enterobacteriaceae* (45.8%), tigecycline resistant *Enterobacteriaceae* (39.0%), and netilmicin resistant *Enterobacteriaceae* (67.2%) Also, this study found a high prevalence for colistin and tigecycline (22.2%) as well as colistin and netilmicin (31.7%) resistance. Colistin and tigecycline combination was observed to be more effective. Among the isolates, *Shigella dysenteriae* showed the highest resistance while isolates obtained from blood cultures were observed to have the highest resistance to colistin, tigecycline, and netilmicin. Among the risk factors that contribute to colistin, non-completion of antibiotics was found to be more significant ($p = 0.039$ and an odds ratio of 0.324 - 0.972 at 95% confidence interval). Our finding is significant in that colistin resistance was determined to be present among populations that have not been exposed to colistin therapy.

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The authors declare no conflicts of interest.

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