



Distribution of Some Antibiotics Resistance Genes in Multi-drug Resistant *E. coli* Isolates from the Urogenitals of Women in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author JAK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EGN and MNA managed the analyses of the study. Author EGN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The problem of antibiotics resistance has assumed a global emergency status. Whereas Multidrug Resistant (MDR) *E. coli* infection is common among human population in Port Harcourt metropolis of Nigeria, the genetic background of *E. coli* isolates in our locality is not well elucidated, hence this study.

Study Design: This was a randomized study of women, with indications of Urogenital infections, attending Braithwaite Memorial Specialist Hospital (BMSH) Port Harcourt, Nigeria between July and December, 2017.

Methodology: Ninety-Seven (97) samples comprising of urine, high vaginal swabs, end ocervical swabs were collected from patients to assess for the presence of some antibiotic resistance genes in multi-drug resistant *E. coli*. Samples were processed following standard microbiological protocols. Antimicrobial susceptibility test was performed on all *E. coli* isolates. Following this, all

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Multiple Drug Resistant *E. coli* were subjected to polymerase chain reaction (PCR) method for the detection of some antibiotic resistance-encoding genes- SHV, CTX-M, TEM.

Results: Seventy-three (73) isolates, including 36 *E. coli*, were recovered from all the clinical specimens. Twenty-four (24) *E. coli* isolates were found to be multi-drug resistant. Sulphydryl Variable (SHV) was the most frequent resistant gene and was detected in 15 isolates. This was followed by Cefotaximase (CTX-M) in 10 isolates and Temoniera (TEM) in 5 isolates. Some isolates harboured more than one resistance gene. About 20% of the isolates harboured SHV/CTX-M; 2.5% harboured CTX-M/TEM, while no target was detected in one isolate.

Conclusion: This present study revealed that most *E. coli* isolates from the urogenitals of women within our locality, possess the ESBL genes which confers on them the Multidrug resistant status and this is a major challenge to maternal health.

Keywords: Antibiotic resistance; MDR, *E. coli*; resistance genes; urogenital infections.

1. INTRODUCTION

Antibiotics are used to treat diseases or infections caused by bacteria. They work by interfering with one of the processes vital to the survival of invading bacteria, such as the formation of the cell wall [1]. There are four mechanisms that are used by bacteria to cause resistance to antimicrobial agents, enzymatic inactivation of the drug, alteration of target sites, reduced cellular uptake and extrusion by efflux [2,3]. It is a known fact that antibiotic resistance crisis is driven by misuse or abuse of antimicrobial agents, as well as lack of new drug development by pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements [4-7]. Antibiotic resistance has threatened the success of medical interventions at all levels of healthcare and creates a set of specific challenges for clinical, therapeutic and public health interventions with local, national and global dimension [8].

Escherichia coli is a bacterial organism that belongs to the family Enterobacteriaceae. *E. coli* is one of the main causes of both nosocomial and community acquired infections in humans. The organism is therefore of clinical importance and can be isolated from various clinical specimens. It is one of the organisms most frequently isolated from blood [9] and from the urogenitals of females, causing various morbidities. It has been observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment [10]. This therefore demands the need for periodic screening of common bacterial pathogens for their antibiotic susceptibility profiles in different communities. According to Aibinu et al. [11], *E. coli* is highly resistant to ampicillin, amoxicillin, tetracycline and

trimethoprim - sulfamethoxazole. The widespread occurrence of drug resistant *E. coli* and other pathogens in our environment has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational prescription programs, making policy decisions and assessing the effectiveness of both [12]. Whereas reasonable information on *E. coli* infection and resistance profile among human population in Port Harcourt metropolis of Nigeria are available, the genetic background of *E. coli* isolates in our locality is not well elucidated, hence this study. The aim of this study was to detect some antibiotic resistance genes (SHV, CTX-M, TEM) in Multi-Drug Resistant *E. coli* isolates from the urogenitals of women in Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Study was carried out in Braithwaite Memorial Specialist Hospital (BMSH), a tertiary care hospital, in Port Harcourt metropolis, Nigeria.

2.2 Ethical Consideration

Ethical approval was obtained from the Braithwaite Memorial Specialists Hospital, Ethical Committee.

2.3 Sample Collection and Processing

Ninety-Seven (97) clinical samples comprising of urine, high vaginal swabs, endocervical swabs were collected from adult females attending the hospital, who were not hospitalized and who had not visited a hospital facility in at least four previous weeks. The samples were inoculated

onto blood agar, MacConkey agar and Cysteine lactose electrolyte deficient (CLED) agar, incubated at 37°C for 24- 48 hours and isolates identified according to method by Cheesbrough [13]. Confirmed *E.coli* isolates were preserved in the refrigerator at 4-8°C and in the freezer at -70°C for antimicrobial susceptibility testing and Molecular analysis respectively.

2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out on the isolates using the disk diffusion method of Kirby-Bauer [14]. Standardized quantity of the isolate matched with 0.5 MacFarland solution was uniformly spread over a Mueller Hinton agar plate. Several filter paper disks impregnated with specific concentration of the selected chemotherapeutic agents were placed on the agar surface and incubated for 18-24 hours and observed for zones of inhibition. The following antimicrobial agents were tested: Gentamicin (10 µg), Nalidixic acid (30 ug), Ofloxacin (10 ug), Ceftazidime (30 ug), Cefixime (30 ug), Cefuroxime (30 ug), Ciprofloxacin (5 ug), Augmentin (30 ug), Ampicillin (30 ug), Ceporex (10 ug), Cotrimoxazole (30 ug), Nitrofurantoin, Cloxacilin, Devixime, Streptomycin (30 ug). Oxoid single disc were used together with Optu/Maxi multiple discs. Results were interpreted as recommended by Clinical Laboratory Standard Institute [15]. Multi-Drug resistance was defined as Organisms resistant to three or more classes of antibiotics.

2.5 Molecular Analysis

The molecular analysis for resistance genes (SHV, CTX-M and TEM) of Multidrug-Resistant *E. coli* isolates was done at the Molecular Biology Laboratory, Niger Delta University (NDU) Bayelsa State, Nigeria.

2.5.1 DNA extraction using boiling method

Pure isolates of *E. coli* were streaked in Luria Betani (LB) broth and were grown overnight. Following this, 2 ml pure 24 hrs broth was transferred into sterile PCR eppendorf tubes properly labelled and centrifuged at 10000 G for

3 minutes; the supernatant was decanted and 1 ml physiological saline was added to the deposit and vortexed. This was centrifuged and decanted again to remove debris. Another 1 ml of normal saline was added to the tubes and incubated in a heating bath for 20 mins at 95°C. The tubes were allowed to cool in a freezer for about 4 minutes and the supernatant were pipetted into another set of tubes for DNA quantification and PCR.

2.5.2 DNA quantification

DNA templates were subjected to quantitative analysis to know the number of copies of DNA present in each isolate. Supernatant from the extracted bacterial DNA was carefully pipetted onto a table top Nano Drop with pre-installed Nano Drop software to obtain the purity and quantity of the DNA.

2.5.3 Amplification of SHV, CTX-M and TEM genes

The SHV, CTX-M and TEM genes were amplified using an applied biosystem thermal cycler at final reaction volume of 20 ul containing master mix (Taq polymerase, dNTPs, MgCl and Buffer), DNA template, Primers, and Water.

2.5.3.1 SHV resistant genes

Forward and Reverse primers for SHV genes were SHV-F- ATGCGTTATATTCGCCTGTG and R-TTAGCGTTGCCAGTGCTC respectively at a PCR concentration of 0.5 ml. The extracted DNA was used as template and amplified for 30 cycles. The PCR temperature condition for SHV genes was set at initial denaturation of 94°C for 5 minutes, denaturation 94°C for 60 seconds, annealing temperature at 52°C for 20 seconds, extension was 72°C for 7minutes.

2.5.3.2 CTX-M resistant genes

Forward and Reverse primers for CTX-M genes were CTX-M-F-GTTACAATGTGTGAGAAGCAG and R-CCGTTTCCGCTATTACAAA

Table 1. Frequency of isolates in clinical specimen in females attending BMSH

| Specimen | <i>E. coli</i> | <i>Klebsiella sp</i> | <i>Proteus sp</i> | <i>Staphylococcus aureus</i> | Total |
|-------------------|----------------|----------------------|-------------------|------------------------------|-------|
| Urine | 26 | 5 | 6 | 18 | 55 |
| High vaginal swab | 7 | 2 | 1 | 4 | 14 |
| Endocervical swab | 3 | 0 | 0 | 1 | 4 |
| Total | 36 (49.3) | 7(9.6) | 7(9.6) | 23 (31.5) | 73 |

Crespectively at a PCR concentration of 0.5 ml. PCR temperature condition for CTX-M gene was set at initial denaturation temperature of 94°C for 5 minutes, denaturation 94°C for 60 seconds, annealing temperature at 54°C for 45 seconds, extension 72°C for 2 minutes. Amplification was for 30 cycles.

2.5.3.3 TEM resistant genes

Forward and Reverse primers for TEM were TEM-F-ATAAAATTCTTGAAGACGAAA and R-GACAGTTACCAATGCTTAATCA respectively at a PCR concentration of 0.5 ml. PCR temperature condition for TEM genes was set at initial denaturation temperature of 94°C for 5 minutes, denaturation at 95°C for 30 seconds, annealing temperature of 51°C for 60 seconds, extension at 72°C for 2 minutes and final extension at 72°C for 5 minutes. Amplification was for 30 cycles.

2.5.3.4 Agarose gel electrophoresis

One gram (1 g) of Agarose was dissolved in 100 ml of TBL and microwaved for 3 minutes. The molten agarose was poured into moulds containing combs and allowed to set. The combs were carefully removed to obtain wells. 2 ul DNA template (product of amplification) from each isolate was pipetted into the wells and allowed to migrate for 25 minutes, stained with ethidium bromide (mutagen) for visibility and viewed with

an ultraviolet trans-illuminator (Gel doc 2000, Bio RAD). The band sizes of the DNA were determined using a mass ruler high range DNA ladder.

2.6 Statistical Analysis

The data generated from the study was analyzed using the Statistical Package for Social Sciences (SPSS) version 21. P-values of ≤ 0.05 were accepted as significant results.

3. RESULTS

Ninety-Seven (97) clinical samples comprising of urine (73), high vaginal swabs (18) and endocervical swabs (6) were collected from adult females attending the BMSH, Port Harcourt. Table 1 shows the frequency of the isolates. *E. coli* was the highest occurring with 49.3%, followed by *Staphylococcus aureus* 23(31.5%).

The susceptibility of the 36 isolates of *E. coli* showed that 12 were susceptible to all antibiotics used and 24 isolates were highly resistant to cefuroxime, cefixime, ceftazidime, augmentin, gentamycin, ceporex and least resistant to ofloxacin (Table 2).

The occurrence rate of Multi-Drug Resistance in the *E. coli* isolates was 24 (66.67%) while 12(33.33%) was Non-MDR as shown in Table 3.

Table 2. Antimicrobial susceptibility of *E. coli* from clinical specimens

| Class of antimicrobial agent | Antimicrobial agent tested | No. susceptible (%) | No. intermediate (%) | No. resistance (%) |
|------------------------------|----------------------------|---------------------|----------------------|--------------------|
| Floroquinolone | Ceporex (Cep)10 ug | 4(11.1) | 12(33.3) | 20(55.6) |
| Monobactam | Augmentin (AU)30 ug | 0(0.00) | 8(22.2) | 28(77.8) |
| Floroquinolone | Ofloxacin (OFX)10 ug | 17(47.2) | 7(19.4) | 12(33.3) |
| Aminoglycoside | Gentamycin (GN)10 ug | 11(30.6) | 9(25.0) | 16(44.4) |
| Cephalosporin | Ceftazidine (CAZ)30 ug | 3(8.3) | 15(41.7) | 18(50.0) |
| Cephalosporin | Cefuroxime (CFX)30 ug | 6(16.7) | 18(50.0) | 12(33.3) |
| Floroquinolone | Ciprofloxacin(CPX)5 ug | 10(27.0) | 12(33.3) | 14(38.9) |
| Floroquinolone | Nitrofurantoin (Nit)30 ug | 13(36.1) | 10(27.0) | 13(36.1) |
| Penicillin | Cloxacin(CXC)10 ug | 0(0.00) | 14(38.9) | 22(61.1) |
| Cephalosporin | Devixime(DXM)30 ug | 11(30.6) | 13(36.1) | 12(33.3) |
| Cephalosporin | Cefixime (CXM)10 ug | 3(8.3) | 12(33.3) | 21(58.3) |
| Floroquinolone | Nalidixic Acid NA)30 ug | 9(25.0) | 10(27.0) | 17(47.2) |
| Floroquinolone | Pefloxacin (Pef)10 ug | 10(27.0) | 6(16.7) | 20(55.6) |
| Aminoglycoside | Streptomycin (S)30 ug | 9(29.0) | 8(22.2) | 19(52.8) |
| Antimetabolite | Septtrin (Sept)30 ug | 7(19.4) | 12(33.3) | 17(47.2) |
| Aminoglycoside | Ampicillin (Pn)10 ug | 4(11.1) | 6(16.7) | 26(72.2) |

Fig. 1 shows Percentage Distribution of SHV, CTX-M and TEM Genes in 24 *E. coli* isolates. SHV was the most frequent resistant gene and was detected in 15 isolates. This was followed by CTX-M in 10 isolates and TEM in 5 isolates. Some isolates harboured more than one resistance gene. About 20% of the isolates harboured SHV/CTX-M; 2.5% harboured CTX-M/TEM, while no target was detected in one isolate.

4. DISCUSSION

Out of 73 isolates from all the urogenital samples, *E. coli* was the most frequently isolated organism (49.3%), followed by *Staphylococcus aureus* (31.5%). *E. coli* remains a leading agent of urogenital infection and is responsible for over 80% of all urinary tract infections, especially in

the females due to the close proximity of their urethra in the urinary tract. Other risk factors that have been adduced include short urethra and behavioural factors such as delay in micturition, sexual activity and the use of spermicides which promote colonization of the periurethral area [16]. In the susceptibility pattern of 36 *E. coli* isolates, 24 isolates were multi-resistant to common groups of antibiotics tested. Isolates were more resistant to the monobactam- Augmentin (77.8%), the aminoglycosides and the cephalosporins. The problem of antibiotic resistance and the factors that promote them, moreso among *E. coli*, is a global one and well reported [9,11,17-19]. Different strains of *E. coli* may harbour or acquire different sets of antibiotics-resistance encoding genes through mobile genetic elements such as plasmids or through spontaneous mutation.

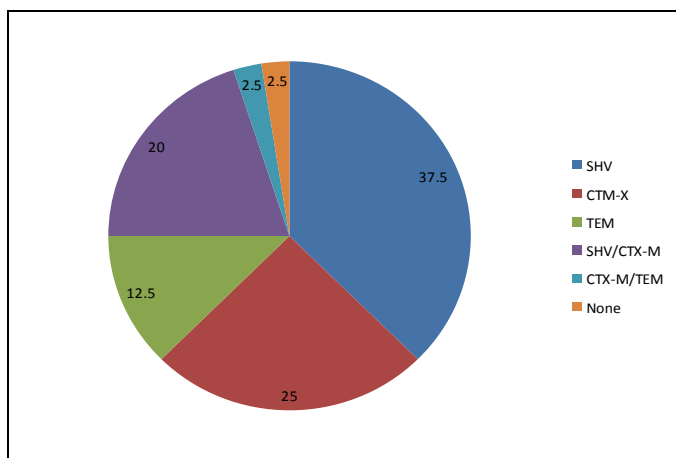


Fig. 1. Percentage distribution of SHV, CTX-M and TEM genes in 24 *E. coli* isolates

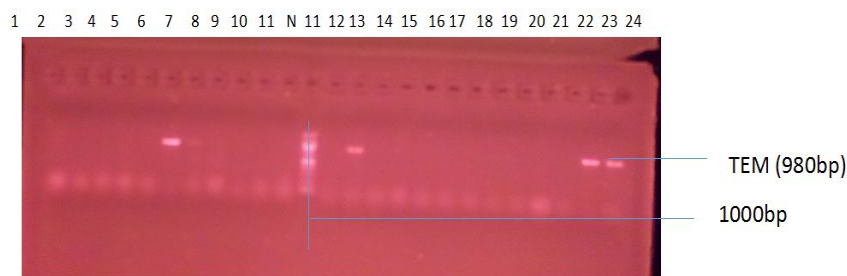


Plate 1. Gel electrophoresis of amplified TEM gene

Lanes 6, 7, 12, 22 and 23 showed the TEM gene band at 980 bp while 1-5, 8-11, 13-21 and 24 were negative for TEM. Lane N represents the 100 bp molecular ladder

Table 3. Occurrence of multidrug resistance (MDR) among *E. coli* isolates

| Category | Number |
|---------------------------------|-------------|
| Multi Drug Resistance (MDR) | 24 (66.67%) |
| Non Multi Drug Resistance (MDR) | 12 (33.33%) |
| Total | 36 (100.0%) |

Read & Woods [20] observed that resistant genes can be inherited from relatives or can be acquired from non-relatives on mobile genetic elements such as plasmids and can also allow antibiotic resistance to be transferred among different species of bacteria. Viswanathan, [21] further reported that sub-inhibitory and sub-therapeutic antibiotic concentrations can promote the development of antibiotic resistance by supporting genetic alterations such as changes in gene expression, horizontal gene transfer and mutagenesis which in turn increases virulence and spread.

In the present study, the resistance genes- SHV, CTX-M, and TEM which are all responsible for ESBL production, were detected in 23 of the 24 *E. coli* isolates tested (Fig. 1). SHV was the most frequent resistance gene and was detected in 15 isolates, predominating the CTX-M and TEM genes. This finding corroborates that of Agi et al. [22] who reported a 100% occurrence rate of SHV among the isolates studied, including *E. coli*. This finding further agrees with Eflakhar et al. [23] who also reported that SHV exceeded CTX-M and TEM. The result obtained however, disagreed with results of studies by Shahid et al. [24] in which CTX-M exceeded TEM and by Yazdi et al. [25] in which TEM exceeded SHV and CTX-M. In a Chinese study, TEM gene predominated followed by SHV. A report from Canada showed SHV as the main gene and in South America and Turkey, it was reported that CTX-M was the most prevalent B-lactamase among ESBL producing *E. coli*. It was also observed from this study, the occurrence of more than one beta lactamase-encoding genes within the same isolate. About 20% of the isolates harboured SHV/CTX-M while 2.5% harboured CTX-M/TEM which probably accounts for the high rate of multidrug- resistance among the *E. coli* isolates. Antibiotics resistance patterns have been observed to vary from one geographical location to the other and even from facility to facility. These may be due to the high mobility of encoding genes.

5. CONCLUSION

This present study revealed that most *E. coli* isolates from the urogenitals of women within our locality, possess the ESBL genes which confers on them the Multidrug resistant status and this is a major challenge to maternal health. Further studies on molecular epidemiology of *E. coli*, in this study location is advocated. This will enhance surveillance information as well as aid

infection control guidelines, policies and planning.

CONSENT

It is not application.

ETHICAL APPROVAL

Ethical approval was obtained from the Ethical Committee, Braithwaite Memorial Specialists Hospital, Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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