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Multidrug Resistance And Susceptibility Profile of Outpatient Escherichia Coli Clinical Isolates

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Abstract

Background: *To gain better understanding about the impact of antibiotic selection pressure on antimicrobial resistance (AMR) in Nigeria, it is important to conduct and review from time to time the susceptibility profile of clinically important bacteria including Escherichia coli. This study investigated the susceptibility and multidrug resistance profile of E. coli isolates from urine samples of patients who received outpatient's medical services in Abakaliki.*

Methods: *A total of 50 non-duplicate clinical isolates of E. coli from the microbiology laboratory section of a tertiary hospital in Abakaliki, Nigeria was recruited for this study. Susceptibility studies were determined using amoxicillin (20 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nitrofurantoin (300 µg) and erythromycin acid (15 µg) by the modified Kirby–Bauer disk diffusion method. All susceptibility studies were carried out as per the guidelines of the Clinical Laboratory Standard Institute (CLSI). Multidrug resistance was evaluated by the multiple antibiotic resistance index (MARI) calculation.*

Results: *The E. coli isolates showed reduced susceptibility to gentamicin (75%), ciprofloxacin (85%), nitrofurantoin (95%), amoxicillin (100%), erythromycin (100%) and ceftazidime (95%). A total of 12 E. coli isolates were multidrug resistant to clinically important antibiotics in the classes: cephalosporins, macrolides, aminoglycosides, fluoroquinolones, penicillins and nitrofurantoin.*

Conclusion: *We report a high rate of antibiotic resistance of outpatients E. coli isolates which concurrently showed multidrug resistance to important antibiotic classes. These findings have clinical significance and provide a benchmark for future studies on the susceptibility pattern of clinical isolates in Nigeria. This preliminary study reiterates the need to reinvigorate antibiotic stewardship in our local hospitals so as to preserve the clinical efficacy of available antibiotics since it takes years for a new antibiotic to be developed.*

Keywords: One Health; Gram negative bacteria; Antimicrobial resistance; Bacterial infection.

Introduction

Antimicrobial resistance (AMR) makes empirical treatment of bacterial infection difficult. AMR is the reduced response of pathogenic microbes that allows them to change and resist the antimicrobial onslaught of potent antibiotics. When AMR occurs, infection could last longer than usual, and it also increases the length of hospitalization, as well as makes treatment difficult. Since AMR bacteria and genes can spread between humans, animals and the environment, it is important to employ a One Health approach in tackling issues of AMR, particularly in the developing countries like Nigeria where cases of AMR is increasing and research and funding towards the curbing of AMR as well as policies surrounding ethical and responsible use of antibiotics in clinical and non-clinical milieus is still at its lowest ebb (Ejikeugwu et al., 2022; Ejikeugwu et al., 2013). AMR has become an issue of public health concern (Gootz, 2010). It is a major factor contributing to mortality and morbidity in settings with limited diagnostic facilities and treatment options especially

in the developing world where health systems are still pitiable (Gootz, 2010; Hailemariam et al., 2021). In addition, drug resistance surveillance has revealed that asymptomatic carriers in the community are often colonized with resistant bacteria that subsequently lead to infection (Donskey, 2006). In Nigeria, antibiotics are readily available over the counter in community pharmacies and other approved outlets of sales. This kind of practice where antibiotics are readily available to anyone portends significant setback to the fight against AMR in Nigeria, and could give room for antibiotics to be used irrationally; thus allowing resistant strains to emerge and spread in the general environment through selective pressure. Pathogenic *Escherichia coli* is a common bacterial infection in humans, and accounts for most hospital visits. The therapeutic treatment of *E. coli* infections is threatened by the emergence of strains resistant to clinically important antibiotics. Being an important member of the Enterobacteriaceae family, *E. coli* is an most important pathogenic bacterium in clinical medicine. It has been reported that they acquire a transmissible form of antibiotic resistance genes including genes for extended spectrum beta lactamase (ESBL) and metallo beta lactamase production (MBL) through genetic transfer routes such as conjugation (Molstad et al., 2008). This implies that some groups of powerful antibiotics including carbapenems and cephalosporins, which have been used over the years to treat infections such as urinary tract infections (UTI's), post-operative infections and blood infections are no longer effective to a large extent as they used to be during their first introduction into clinical medicine some decades ago (Molstad et al., 2008). Studies have also shown that the resistance of bacteria to available antibiotics has dramatically risen, and both ESBLs and MBLs have contributed to this phenomenon in recent times, and reducing the clinical efficacy of these antibiotics (Shakil et al., 2009; Wassef et al., 2010; Zhang et al., 2010). Many infections of *E. coli* usually result from eating meat that has come in contact with waste from animal intestines during processing (Hamza et al., 2016; Song et al., 2020). Environments that are heavily contaminated with human or animal wastes are also other important sources of contamination or infection. Owing to the fact that antibiotic resistance is an increasing public health problem that is of global concern, it is vital to check the emergence and spread of resistant pathogens through prompt and accurate detection techniques so as to assuage any outbreak due to them. This research project investigated the susceptibility and multidrug resistance profile of pathogenic outpatients *E. coli* clinical isolates from urine samples in Abakaliki, Nigeria.

Materials and Methods

Ethical Approval: This study was conducted following all relevant national and international guidelines as stipulated in the World Medical Association (WMA) Declaration of Helsinki. The study received ethical approval from the Local Ethics and Research Committee of the Faculty of Sciences, Ebonyi State University, Abakaliki, Nigeria. All the clinical isolates recovered from the microbiology section of the tertiary hospital were handled and processed according to the WMA guidelines on Medical Research involving research on identifiable human materials and data.

Re-characterization of Bacterial Isolates: A total of 50 non-duplicate isolates of *E. coli* were recovered from the microbiology section of the tertiary hospital under study. Each of the isolates were re-characterized using standard microbiology techniques including biochemical testing, cultural techniques and microscopy as was previously described (Ejikeugwu et al., 2013; Cheesbrough, 2006). Discrete strains of the *E. coli* isolates were selected on MacConkey agar and purified on nutrient agar plates, and these were further used for performing the antimicrobial susceptibility studies.

Susceptibility Studies: All *E. coli* isolates were subjected to antibiotic susceptibility studies using selected single antibiotics disks (Oxoid, UK) comprising: amoxicillin (AML: 20 µg), ceftazidime (CAZ: 30 µg), ciprofloxacin (CIP: 5 µg), gentamicin (CN: 10 µg), nitrofurantoin (F: 300 µg) and erythromycin (E: 15 µg). These antibiotics were selected for this study because they are regularly prescribed antibacterial agents that are commonly obtained over-the-counter (OTC) in Nigeria. To perform the susceptibility studies, the single antibiotic disks were placed at a distance of 20mm apart on freshly prepared Mueller Hinton (MH) agar plates (Oxoid, UK) already inoculated with the test *E. coli* isolates already adjusted to 0.5 McFarland's turbidity standards (Ejikeugwu et al., 2022). The susceptibility test plates were incubated at 37°C for 18-24h. The zones of inhibition produced was measured and reported as resistance or susceptible as per the standard antibiotics breakpoints and guidelines of the (Clinical Laboratory Standard Institute, [CLSI] 2018).

Multiple Antibiotic Resistance Index (MARI): MARI of the resistant isolates was determined/calculated according to a previously used protocol using the MARI formular (Ejikeugwu et al., 2022). To calculate MARI, the following formular was used: MARI= Number of antibiotics to which resistance occurred / total number of antibiotics to which the isolates were tested.

Results

Figure 1 show the susceptibility and resistance profile of the clinical isolates of *Escherichia coli* isolates tested against some clinically relevant antibiotics. In this preliminary study, we investigated the susceptibility profile of clinical isolates of outpatients *E. coli* from the microbiology section of a tertiary hospital in Abakaliki, Nigeria. A total of 50 non-duplicate clinical isolates of *E. coli* were re-characterized and subjected to susceptibility studies using single antibiotic disks that are representative samples of commonly prescribed antibiotics in Nigerian hospitals. The *E. coli* strains were completely resistant to amoxicillin (100%) and erythromycin (100%). And this was followed by reduced susceptibility to gentamicin (75%), ciprofloxacin (85%), nitrofurantoin (95%) and ceftazidime (95%).

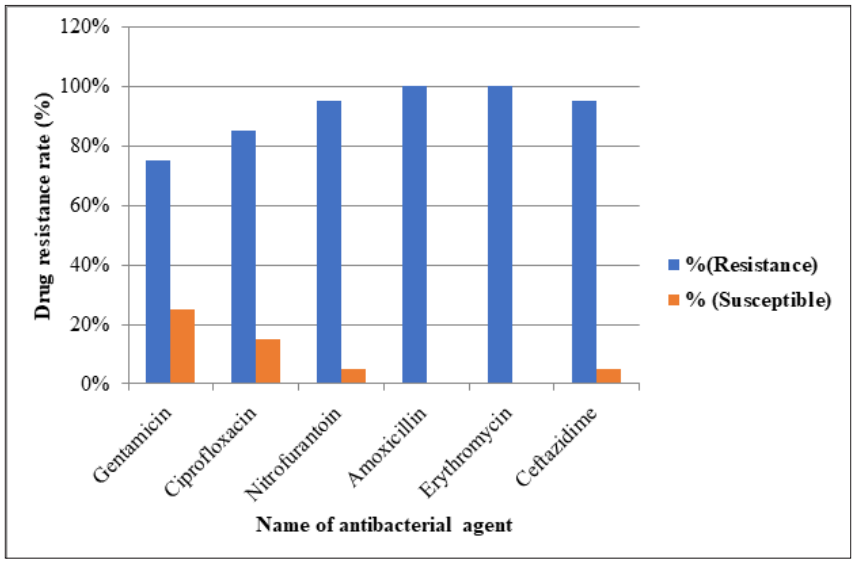

Figure 1: Resistance and susceptibility profile of the outpatients *E. coli* clinical isolates

Table 1 shows the results of the multiple antibiotic resistance index (MARI) profiling carried out on some selected multidrug resistant outpatients *E. coli* isolates investigated in this study. A total of six (6) antibiotics from 5 antibiotics classes (macrolides, cephalosporins, penicillins and aminoglycosides) were screened for multidrug resistance against the test *E. coli* strains in this study. The multidrug resistance index profiling of the resistance isolates in this study revealed several levels of resistance to the tested antibiotics from the 4 different classes of antibiotics used. Out of the 50 outpatients *E. coli* isolates investigated in this study, a total of 12 *E. coli* isolates were found to be multidrug resistant (Table 1). Our results also showed that the highest multidrug resistance rate was recorded against ceftazidime in the cephalosporin family, and this was followed by gentamicin in the aminoglycoside family, erythromycin in the macrolide family and finally, ciprofloxacin in the fluoroquinolone family. On average, all the resistant *E. coli* strains had a MAR profile that ranged between 0.5-0.8, indicating that the test isolates had a high multidrug resistance profile to the tested antibiotics in this study.

Isolate No.	MAR profile	Antibiotics
E3	0.6	CIP, F, AML, CAZ
E12	0.6	CN, CIP, F, CAZ
E20	0.8	CN, CIP, F, E, CAZ
E8	0.6	CN, CIP, F, AML
E31	0.8	CN, F, E, AML, CAZ
E5	0.5	CN, CIP, CAZ
E7	0.6	CN, E, AML, CAZ
E25	0.6	F, E, AML, CAZ
E15	0.8	CIP, F, E, AML, CAZ
E44	0.5	CN, E, AML
E6	0.8	CIP, F, E, AML, CAZ
E18	0.6	F, E, AML, CAZ

Table 1: Multidrug resistance profiles of the outpatients *E. coli* isolates

Discussion

Antimicrobial resistance (AMR) continues to pose a great threat to public health globally. This culminates to serious health problems such as prolonged hospitalization and treatment failures. *Escherichia coli* is an important member of the ESKAPE (Enterococcus, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, *E. coli*) family of bacteria responsible for causing nosocomial infections globally. It is also a common causative agent of bacterial infections and an easily encountered bacterial strain in most microbiology laboratories in hospitals in Nigeria. In this current study, we investigated the susceptibility and multidrug resistance profile of outpatients *E. coli* isolates recovered from urine samples of patients who received outpatient medical services in Abakaliki, Nigeria. From our study, it was discovered that the *E. coli* isolates showed complete resistance to erythromycin (100%) and amoxicillin (100%). Erythromycin is a macrolide and a protein synthesis inhibitor while amoxicillin is a beta lactam agent that inhibits cell wall development in bacteria. Both macrolides and amoxicillin are clinically used for the clinical management and treatment of infections caused by pathogenic *E. coli* and other clinically relevant bacteria. In addition, the *E. coli* isolates showed reduced susceptibility to ceftazidime (95%), nitrofurantoin (95%) gentamicin (75%) and ciprofloxacin (85%). The rates of resistance of the outpatients *E. coli* to the clinically important antibacterial agents used in our study is higher than the resistance rates reported elsewhere - where *E. coli* isolates from routine clinical samples including urine showed lower resistance rates to amoxicillin (34.6%), gentamicin (42.6%), ceftazidime (40.4%) and ciprofloxacin (53.4), as well as to other antibiotics that we did not investigate in our current study (Furlan & Stehling, 2021). In another related study conducted in the United States of America, the *E. coli* isolates recovered from the urine samples of outpatients with uncomplicated urinary tract infections (UTIs) were found to be multidrug resistant and showed varying levels of resistance to antibiotics in the classes: aminoglycosides, cephalosporins, penicillins, carbapenenms, sulphamethoxazoles and nitrofurantoin (Ku et

al., 2023). Nitrofurantoin is an important antibacterial agent used for treating and managing both uncomplicated UTI. The high resistance of the outpatients *E. coli* isolates in our study (95%) is worrisome and calls for urgent action to reverse this trend. In China, a five year study also reported an overall (increasing) antibiotic resistance rate (85%) of *E. coli* isolates recovered from neonatal intensive care units (ICUs) from 2015-2020 [16]. The *E. coli* isolates in this China study which also produced ESBL (extended spectrum beta lactamase) also showed high insensitivity to antibiotics in the classes: cephalosporins, penicillins, aminoglycosides, quinolones and carbapenems (Xiao et al., 2023). Overall, a total of 12 isolates of the outpatients *E. coli* showed multidrug resistance to antibiotics in the classes: aminoglycosides, fluoroquinolones, penicillins, cephalosporins, macrolides and nitrofurantoin. Multidrug resistant *E. coli* have been emergeing and spreading across the globe in both developed and developing economies (Shakil et al., 2009; Zhang et al., 2010; Ku et al., 2023; Tchesnokova et al., 2023). This kind of increase in the multidrug resistance profile of outpatients *E. coli* is a cause for concern as it regards the clinical management and treatment of *E. coli* associated infections in Nigeria. Our study adds to the growing evidence of AMR in both the hospital and non-hospital milieus in Nigeria, and calls for concerted efforts to curb this health menace in a One Health approach. Bacterial resistance to clinically important antibiotics especially last-line antibiotics such as carbapenems and cephalosporins as reported in our study compromises the clinical effectiveness of available drugs and results in poor patients prognosis, long hospitalization and other economic burden.

Conclusions

From our study, a high rate of antibiotic resistance was reported and most of the outpatients *E. coli* isolates showed significant resistance to commonly prescribed antibiotics including nitrofurantoin, erythromycin, ceftazidime, amoxicillin, ciproflocxacin and gentamicin. Regular surveillance and detection is required to provide better guideline about susceptibility profile of clinical isolates of *E. coli* for optimal (empirical) therapy in Nigerian hospitals. While there are varying factors driving AMR in the general environment, we recommend a One Health approach to tackling the issues of AMR in local hospitals in Nigeria, and this should encompass rational antibiotic use in both human and animal health since these lifesaving medications (antibiotics) may be abused beyond our understanding.

Supplementary Materials

Not applicable.

Author Contributions

Conceptualization, RE and CE.; methodology, RE; EO; CE; validation, MA and CE; formal analysis, CE and EO; CA and NU.; investigation, RE; EO; MU; NU; data curation, RE; MU and CE.; writing—original draft preparation, CE, CA and RE; writing—review and editing, CE; MU and MA.; supervision, CE, MU and MA: All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

All methods in this study were carried out in accordance with the guidelines of the World Medical Association (WMA) Declaration of Helsinki for experiments related to data from animal and human sources. The study received ethical approval from the Local Ethics and Research Committee of Ebonyi State University, Nigeria, and all methods were carried out in compliance with the ARRIVE guidelines.

Informed Consent Statement

Inform consent was sought and obtained prior to the processing of the isolates and publication of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included here and are available from the corresponding author on reasonable request.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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