Isolation, Identification, and Antibiogram of Uropathogenic *Escherichia coli* Isolated from Ambulatory Patients with Suspected Cases of Urinary Tract Infection (UTI)

ABSTRACT

Urinary tract infections (UTIs) are prevalent health issue, with *Escherichia coli* being the primary causative agent. The aim of the study was to isolate, identify, and evaluate the antibiotic susceptibility pattern of uropathogens *Escherichia coli* (UPEC) from ambulatory patients with suspected cases of UTI. The primary isolation of the UPEC was done using cystine electrolyte deficient agar and MacConkey agar. Different biochemical test was carried out on the isolates for the purpose of characterization and identification. Antibiotics susceptibility test was carried out using Kirby-Bauer disc diffusion method. A total of 366 urine samples were collected and processed, resulting in the identification of 192 UPEC isolates. Other bacterial isolates identified in this study were *Klebsiella* spp, *Enterobacter* spp, *Acinetobacter* spp, *Citrobacter* spp, *Pseudomonas aeruginosa*, *Proteus* species, *Staphylococcus aureus*, Coagulase negative *Staphylococcus* (CONS), *Enterococcus* spp and *Bacillus* spp. High susceptibility was observed to ciprofloxacin (92.7%) and Levofloxacin (85.4%), while significant resistance was noted for ceftriaxone resistance (19%) and Pandrug resistance (2.1%) was observed in UPEC isolates. The findings highlight the need for continuous monitoring of antibiotic resistance patterns to guide effective UTI treatment strategies and mitigate the spread of resistant UPEC strains.

INTRODUCTION

Uropathogenic *Escherichia coli* (UPEC) is the primary causative agent of urinary tract infections (UTIs), a common infectious disease affecting individuals of all age groups worldwide. UPEC is responsible for up to 70–90% of community-acquired UTIs and a significant proportion of nosocomial UTIs (Kaper *et al*., 2004; Flores-Mireles *et al*., 2015). UTIs are a major public health concern, particularly among women, where anatomical factors predispose them to higher rates of infection. In the elderly, catheterized patients, and individuals with compromised immune systems, UTIs can lead to severe complications, such as pyelonephritis and urosepsis, making the study of UPEC essential to improving clinical outcomes. UPEC is a pathotype of *Escherichia coli*, a Gram-negative bacterium that normally inhabits the gastrointestinal tract as a commensal organism. However, specific strains of *E. coli* have evolved virulence factors that allow them to infect the urinary tract, where they can cause a range of infections from uncomplicated cystitis (bladder infection) to severe upper urinary tract infections (pyelonephritis) (Johnson & Russo, 2002). The pathogenesis of UPEC is complex and involves various mechanisms that allow the bacteria to adhere to, invade, and persist within the urinary tract epithelium.

UPEC's ability to cause disease is largely due to a specialized set of virulence factors that enhance its pathogenic potential. Adhesion to the uroepithelium is one of the important steps in UTI pathogenesis. UPEC expresses multiple adhesive pili (fimbriae), the most well-studied being type 1 pili, which mediate binding to mannose-containing receptors on bladder epithelial cells through the FimH adhesin (Kline *et al*., 2009). This adhesion promotes colonization and facilitates the formation of intracellular bacterial communities (IBCs) within the bladder,

allowing UPEC to evade host immune responses. Additionally, P pili, encoded by the pap operon, enable UPEC to bind to receptors on kidney cells, contributing to the development of pyelonephritis (Spurbeck & Mobley, 2013). Other virulence factors include toxins such as hemolysin (HlyA), which lyses host cells, and siderophores like enterobactin, which allow UPEC to scavenge iron in the iron-limited environment of the urinary tract (Johnson & Russo, 2005).

The process by which UPEC causes UTIs typically begins with the ascent of bacteria from the intestinal reservoir to the periurethral area, followed by entry into the bladder (Foxman, 2010). Once inside the bladder, UPEC adheres to the epithelial cells, triggering an inflammatory response leading to the hallmark cystitis symptoms, such as dysuria and frequent urination. In some cases, UPEC can ascend to the kidneys, leading to pyelonephritis, a more severe infection associated with flank pain, fever, and the risk of systemic infection (Nielubowicz & Mobley, 2010). One of the key features of UPEC pathogenesis is its ability to persist within the urinary tract despite the host immune response. UPEC can invade bladder epithelial cells and form IBCs, where they are shielded from antibiotics and immune clearance (Anderson *et al.*, 2003). This intracellular lifestyle not only contributes to the persistence of the infection but also increases the risk of recurrence. Recurrent UTIs are common in UPEC infections, with some individuals experiencing multiple episodes of infection over their lifetime (Hannan *et al*., 2010).

In recent years, the emergence of antibiotic-resistant UPEC strains has become a significant concern in UTI management. UPEC has developed resistance to many commonly prescribed antibiotics, including fluoroquinolones, trimethoprim-sulfamethoxazole, and beta-lactams (Nicolle, 2019). The spread of multidrug-resistant (MDR) strains, particularly those producing extended-spectrum beta-lactamases (ESBLs), limits the treatment options for UTI patients, often requiring the use of more toxic or expensive antibiotics (better word for toxic) (Shaikh *et al.*, 2015). Surveillance of antibiotic resistance patterns is essential to guide the empirical treatment of UTIs and reduce the prevalence of MDR UPEC strains. Hence, the study seeks to isolate, characterize, and identify UPECs and determine their susceptibility to commonly used antibiotics.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this study was obtained from the Cross River State Health Research Ethics Committee with reference number CRSMOH/HRP/REC/2023/405. The ethical statement was made available to the different hospitals, and their informed consent was also obtained verbally and in writing before the commencement of the study. Confidentiality was maintained by labeling the samples with codes rather than participant names. All methods were carried out under relevant guidelines and regulations (Declaration of Helsinki).

Sample Collection/Processing

Freshly voided 5-10 ml of clean catch midstream urine specimens were collected from ambulatory patients with suspected cases of UTI using a sterile, graduated, wide-mouthed plastic universal urine container. All participants were instructed on how to collect clean catch midstream urine. Also, the female participants were provided with sterile gauze to make front-to-back wiping dry before urination. Specimens were kept at cold chain transportation using ice packs in a cool box after collection. All samples were analyzed immediately (within one hour)

after arrival at the laboratory to ensure that the UPEC in the urine was isolated and to avoid overpopulation of the uropathogens.

Isolation, Characterization, and Identification

Urine samples (0.001 ml) were directly inoculated into cysteine lactose electrolyte deficient (CLED) agar, and MacConkey agar (Oxoid, Ltd), using a sterile standard calibrated wire loop. After 24 hours of aerobic incubation at 37□, the plates were examined macroscopically for morphological appearance as presumptive identification. A colony count of $\geq 10^3$cfu/mL was considered a significant bacterial count. The UPEC isolates were characterized based on colonial and cell morphology, growth on differential and selective media (MacConkey agar, CLED agar, EMB agar), Gram reaction, and biochemical tests. Then identified by comparing their characteristics with known taxonomy using the scheme of Cowan and Steel (1993).

Antibiotic Susceptibility Test

The Kirby-Bauer disk diffusion method described by Bauer *et al.*, 1996, Andy and Okpo, 2018, and Okpo *et al.*, 2023 was used to perform the antibiotic susceptibility test on Mueller-Hinton agar using the following antibiotics: Gentamicin (10µg), Streptomycin (10 µg), Nitrofurantoin (100 µg), Erythromycin (15 µg), Amoxicillin (30 µg), Augmentin (30 µg), Cephalaxin (spell) (30ug), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), and Levofloxacin (5 µg). The result was interpreted according to the criteria of the Clinical Laboratory Standard Institute (CLSI, 2012).

RESULTS AND DISCUSSION

Urinary tract infection (UTI) is among the most common bacterial infections, with *Escherichia coli* (*E. coli*) being the leading uropathogens. Uropathogenic *E. coli* (UPEC) is responsible for the majority of both community acquired and health care-associated UTIs. Timely isolation and identification of UPEC are essential for proper diagnosis and treatment. This study was focused on isolating, identifying and evaluating the antibiotic susceptibility patterns of UPEC from ambulatory patients with suspected UTIs.

In this study, a total pf 366 urine samples were collected from ambulatory patients with suspected cases of UTI and processed for the isolation of UPEC. Out of the 366 samples, 192(57%) yielded bacterial growth typical of *E. coli*. The isolates exhibited characteristic colony morphology on MacConkey agar as pink, lactose-fermenting colonies. Further biochemical tests which include indole production (+), methyl red test (+), voges-proskauer test (-), catalase test (+), motility test (+) and citrate utilization test (-) was used to confirmed (tense)the identity of the isolates as *E. coli*. As reported by several researches, *E. coli* is the number one organism involved in UTI (Yismaw *et al.*, 2012). Several factors may have influenced the prevalent of *E. coli*. *E. coli* is commonly found in the gastrointestinal tract, particularly in the colon (Iseppi *et al.*, 2020), which is anatomically closer to the urethra. This proximity makes it easier for *E. coli* to enter the urinary tract.

Moreso, *E.coli* have some specialized adhesive properties such as fimbriae (or pili) that allow it to adhere to the cells lining the urinary tract, resisting the natural flushing action of urine. This makes it easier for this organism to colorize (spell)the bladder and the urinary system. Furthermore, some strain of *E. coli* produces toxins and other virulence factors that help them invade and survive within the urinary tract. For example, the production of hemolysin can

damage host cell and siderophores allow them to scavenge iron from the host, which is essential for bacterial growth (Cho *et al*., 2015).

The percentage frequency of occurrence of bacterial uropathogens isolated from UTI suspected patients is presented in Figure 1. As seen in the table, *E. coli* had the highest percentage frequency of occurrence (57%) followed by *Klebsiella* spp (12.2%). Other uropathogens with their percentage frequency of occurrence were *Enterobacter* spp 4.2%, *Acinetobacter* spp 3.3%, *Citrobacter* spp 2.4%, *P. aeruginosa* 3.9%, *Proteus* spp 2.2%, *Staphylococcus* spp 5.6%, coagulase-negative staphylococcus (CoNS) 6.5%, *Enterococcus* spp 2.1% and *Bacillus* spp having the least percentage frequency of occurrence (0.6%). This study revealed that gram-negative bacteria are more common cause of UTI than gram-positive bacteria. Similar findings have been reported in a study conducted by Addis *et al*. (2021). The potential of gram-negative bacteria to cause UTI is because gram-negative bacteria have an outer membrane that makes them resistant to certain host immune responses, such as complement-mediated killing. This membrane also helps them resist some antibiotics, making them more persistent in the urinary tract. Also, the outer membrane of gram-negative bacteria contains lipopolysaccharides, which make their cell wall more hydrophobic. This allows them to better resist the flushing action of urine and adhere to the urinary tract's mucosal surface (Yismaw *et al*., 2012). In contrast, Gram-positive bacteria like *Staphylococcus* spp and *Enterococcus faecalis* do cause UTIs but are less common, largely because they lack many of these specific virulence factors and are less adapted to the urinary environment compared to gram-negative bacteria.

The antibiotic susceptibility profiles of the 192 confirmed UPEC isolates were determined using the Kirby-Bauer disc method. The results showed varying degrees of susceptibility and resistance to the tested antibiotics. The UPEC were subjected to ten (10) antibiotics belonging to five (5) different classes of antibiotics (Quinolones, Cephalosporin, Aminoglycoside, Penicillin, and Macrolides). Most of the UPEC were observed to be sensitive to Ciprofloxacin, Levofloxacin, Gentamicin, Nitrofurantoin, Erythromycin and Augmentin. A high level of resistance was observed with cephalexin and ceftriaxone and moderate resistance to amoxicillin and streptomycin as presented in Table 1.

Of the 192 UPEC isolates, 93(48.4%) exhibited multidrug resistance (MDR), defined as resistance to three or more classes of antibiotics, 36 (19%) exhibited extensively drug resistance (XDR) and 4 (2.1%) exhibited Pandrug resistance (PDR). The ability to exhibit resistance by UPEC may be due to several biological and environmental factors that promote the development and spread of antimicrobial resistance. *E. coli* can acquire resistance genes from other bacteria through horizontal gene transfer (HGT), which include mechanisms such as conjugation, transformation, and transduction. This enables the transfer of plasmids, transposons, and integrons that carry resistance genes between different bacterial species and strains (Partridge *et al.*, 2018). The widespread and often inappropriate use of antibiotic in healthcare, agriculture, and animal husbandry creates selective pressure. This favours the survival of resistant UPEC strains, allowing them to proliferate while sensitive strains are eradicated. Over time, this selective pressure contributes to MDR, XDR, and PDR development. Also UPEC strains can produce enzymes like extended-spectrum β-Lactamases (ESBLs), Ampc β-lactamases or carbapenemases that can break down a wide range of β-lactam antibiotics. This also contributes to MDR, XDR, and PDR (Bush and Bradford, 2020). Figure 2 shows the percentage of MDR, XDR and PDR uropathogenic *E. coli* isolated in this study.

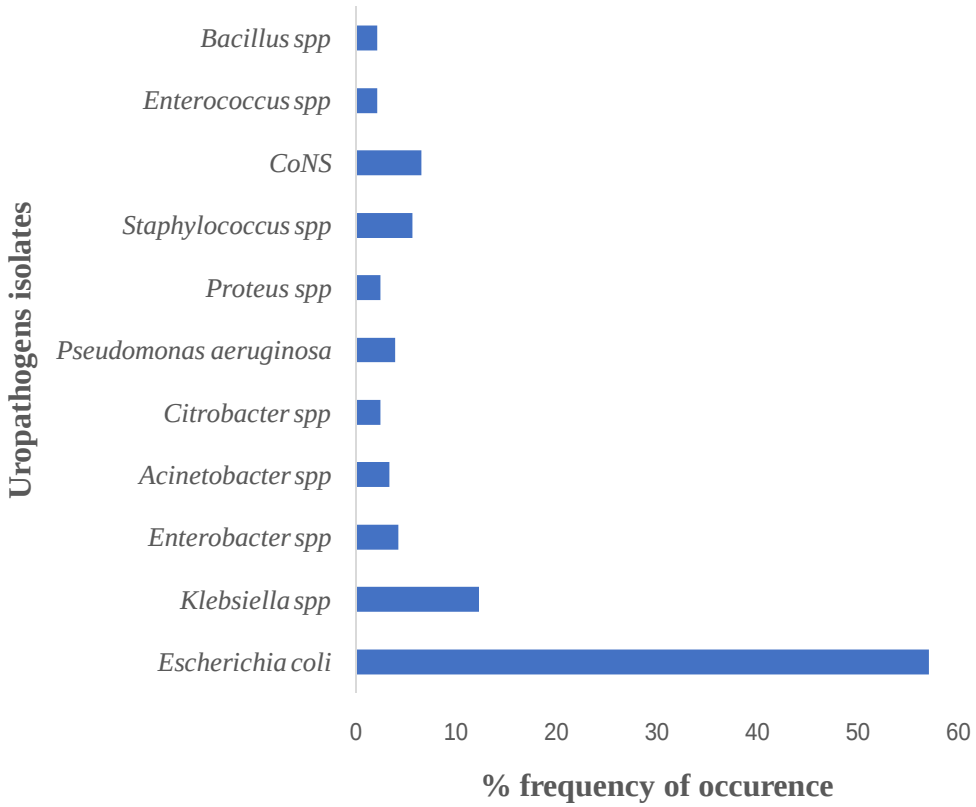

Figure1: Percentage frequency of bacterial uropathogens isolated from UTI-suspected patients

Table 1: Antibiotic susceptibility profile of UPEC isolates

Antibiotics	Sensitive	Resistance
Gentamicin (10µg)	151 (78.6%)	41 (21.4)
Streptomycin (10µg)	161 (83.9%)	31 (16.1%)
Nitrofurantoin (10µg)	127 (66.1%)	65 (33.9%)
Erythromycin (15µg)	134 (69.8%)	58 (30.2%)
Amoxicillin (30µg)	152 (79.2%)	40 (20.9%)
Augmentin (30µg)	137 (71.4%)	55 (28.6%)
Cephalexin (30µg)	82 (42.7%)	110 (57.3%)
Ceftriaxone (30µg)	28 (14.6%)	164 (85.4%)
Ciprofloxacin (5µg)	178 (92.7%)	14 (7.3%)
Levofloxacin (5µg)	164 (85.4%)	28 (14.6%)

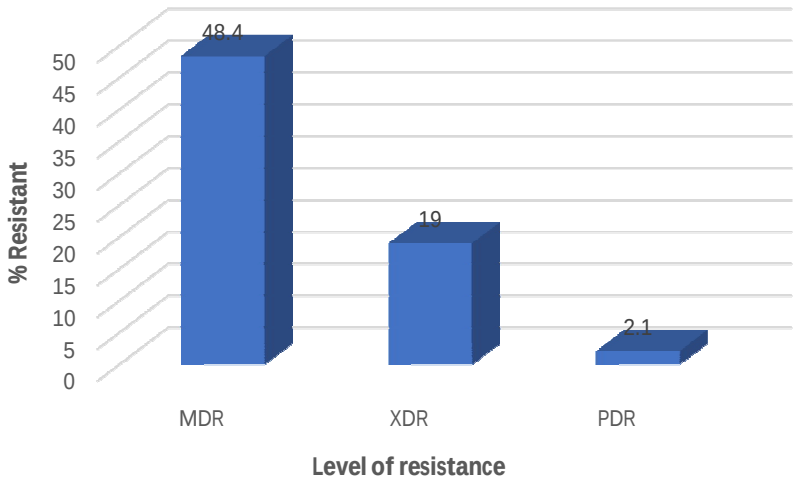

Figure 2: Leve of resistance of UPEC isolates

CONCLUSION

This study highlights the prevalence of UPEC among ambulatory patients with suspected UTIs and demonstrates the growing concern of antibiotic resistance in the management of UTIs. Continuous monitoring of antimicrobial susceptibility patterns is essential to guide effective treatment protocols and limit the spread of resistant strains. Finally, research into the development of new antibiotics should be encourage(tense) as several microorganisms have developed resistance to all the available antibiotics.

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