

Antibiotic Susceptibility Pattern of *Escherichia coli* Isolated from Out-patient Individuals Attending the University College Hospital (UCH), Ibadan, Nigeria

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Abstract

A retrospective review of culture media of urine, ascitic fluid, sputum, tracheal aspirate, wound biopsy, antral washouts and blood was taken. The aim of this study was to determine the prevalence and antimicrobial susceptibility of *Escherichia coli* from clinical sources. A total of 32 samples were analyzed for isolation and identification of bacteria and antimicrobial susceptibility testing. *Escherichia coli* were isolated from 14 (43.75%) samples. The *E. coli* co-infection was highly implicated in urine specimen (21.42%). The *E. coli* isolates showed resistance to ciprofloxacin (92.86%), cotrimoxazole (92.86%) and ceftriazone (78.58%). Lower susceptibility was observed with ofloxacin (28.57%). Indiscriminate use of antibiotics should be discouraged. Regular hygiene methods should be advocated among community dwellers and hospital personnel.

Keywords: Antibiotics resistant; Hospital; *Escherichia coli*; Urinary tract infections; Patients

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Introduction

Escherichia coli is the most common cause of urinary tract infections (UTIs) in humans and is a leading cause of enteric infections and systemic infections [1]. The systemic infections include bacteremia, nosocomial pneumonia, cholecystitis and infectious arthritis. *E. coli* is also a leading cause of neonatal meningitis [2].

Escherichia coli and related bacteria constitute about 0.1% of gut flora [3]. Faecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease also, *E. coli* majorly is responsible for a wide variety of hospital and community onset infections, affecting patients with normal immune systems as well as those with pre-existing conditions [4]. They often comprise most Gram negative bacteria found in clinical laboratories including the vast majority of urinary, blood culture and peritoneal isolates. Several variants or pathotypes of *E. coli* have been described as causing infections of the gastrointestinal system while other pathotypes cause infections outside the gastrointestinal system [5]. The pathogenic ability of *E. coli* is largely affordable by the flexible gene pool through the gain and loss of materials [6,7].

Antibiotic resistant isolates, especially those that are fluoroquinolones resistant and those producing extended-spectrum β -lactamases have increased significantly during the 2000's and in certain areas while many nosocomial and community-acquired *E. coli* are now resistant to several important antimicrobials [4]. A wide range of antimicrobial agents effectively

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inhibit the growth of *E. coli*. The β -lactams, fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole are often used to treat community and hospital infections associated with *E. coli* [3]. Clinical studies of antimicrobial therapy and the outcome of patients infected with carbapenemase-producing *E. coli* compared with patients infected with susceptible strains are limited and suggest worse clinical outcomes for patients with infections due to resistant isolates [8].

This study was designed to isolate, identify, and determine the susceptibility index of *E. coli* from clinical sources obtained from patients at the University College Hospital (UCH) Ibadan, Nigeria.

Materials and Methods

Sample collection

Clinical samples from patients diagnosed with various infections were obtained from the Microbiology Laboratories of the Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria. The samples were collected on prepared agar slants.

Sample population

A total of 32 samples were taken from the Department of Microbiology and Parasitology of the University College Hospital (UCH), Ibadan, Nigeria and transferred to the Laboratories of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria for subsequent culturing and biochemical tests.

Growth on Eosin Methylene Blue Agar (EMB)

Pinkish colonies on MacConkey agar were transferred on EMB agar. Greenish metallic sheen colonies on EMB were identified after incubation for 18-24 hours at 37°C. They were stored for future use.

Catalase test

The ability to produce catalase by the isolates was determined using hydrogen peroxide.

Bacterial Isolation and Identification

Culture plates from Deoxycholate agar (Oxoid, UK), MacConkey agar (Oxoid), nutrient agar, blood agar were used. The swab sticks used for the collection of the samples were streaked directly on the labeled agar plate and incubated at 37°C for 24 h. After incubation, cultures were examined for significant growth. Biochemical test were performed to identify microbes that could not be characterized by morphology. Biochemical tests applied were standard catalase test, citrate utilization, oxidase, Voges-Prokauer, indole production, motility, sucrose, maltose, lactose, nitrate reduction and mannitol [9].

Antibiotic Susceptibility Test

In vitro susceptibility of the isolates against antimicrobial agents was determined by the standard disc diffusion procedure. The following antibiotic discs were used: augmentin (AUG; 30 μ g), ceftriazone (CRO; 30 μ g), nitrofurantoin (NIT; 300 μ g), gentamycin (GEN; 10 μ g), ofloxacin

(OFL; 5 μ g), amoxicillin (AMX; 25 μ g), ciprofloxacin (CPX; 5 μ g), tetracycline (TET; 30 μ g), pefloxacin (PFX; 5 μ g). The *E. coli* isolates were inoculated in nutrient broth and incubated at 37°C for 24 hours. The inoculum was transferred into the Mueller-Hinton agar plates. Antibiotic discs were carefully placed on the plates. Plates were incubated at 37°C for 18 to 24 hours. The zones of inhibition were measured, recorded and interpreted according to the Clinical Laboratory Standard Institute provided [10].

Results and Discussion

Out of the 32 clinical isolates in this investigation. 14 isolates were identified as *Escherichia coli*.

Table 1 shows the percentage distribution of the *E. coli* in relation to age and sex. Male individuals which fall within the age range 61-70 constituted highest *E. coli* infection susceptible individuals of 3 (21.43%) followed by 2 (14.28%) females with age range of (0-10). The least *E. coli* infected persons were within the age range of (11-20) and (21-30) showing percentage constitution of (7.14%) respectively.

Table 1 Distribution of *E. coli* infections in relation to age and sex.

| Age range | Isolate code | Total number of Patient samples | Number of % Male | Number of % Female |
|-----------|--------------|---------------------------------|------------------|--------------------|
| 0-10 | CLS3 | 2 | - | 14.28 |
| | CLS4 | | | |
| 11-20 | CLS11 | 1 | - | 7.14 |
| 21-30 | CLS13 | 1 | 7.14 | - |
| 31-40 | CLS9 | 2 | 14.28 | - |
| | CLS14 | | | |
| 41-50 | CLS2 | 3 | 14.28 | 7.14 |
| | CL10 | | | |
| | CLS12 | | | |
| 51-60 | CLS1 | 2 | 7.14 | 7.14 |
| | CLS6 | | | |
| 61-70 | CLS5 | 3 | 21.43 | - |
| | CLS7 | | | |
| | CLS8 | | | |

Table 2 Distribution of *E. coli* infection case histories.

| Case histories/ <i>E. coli</i> infection | Isolate codes | Total number % |
|--|---------------|----------------|
| Wound sepsis | CLS1 | 7.14 |
| Flame burns | CLS2 | 7.14 |
| Chronic sinusitis | CLS3 | 7.14 |
| Retroviral disease on ART | CLS4 | 7.14 |
| Urinary tract infection | CLS5 | 14.28 |
| Urinary tract infection | CLS9 | - |
| Jaundice | CLS6 | 7.14 |
| Chronic liver disease | CLS7 | 7.14 |
| Dysuria | CLS8 | 7.14 |
| Lower respiratory tract infection | CLS10 | 7.14 |
| Cough | CLS11 | 7.14 |
| Multiple myeloma | CLS12 | 7.14 |
| Tuberculosis | CLS13 | 7.14 |
| Known Hbs with Haemolytic crisis | CLS14 | 7.14 |

Table 2 showed the distribution of *E. coli* infection in polymicrobial association and variable medical diagnosis. Urinary tract infection 2 (14.28%) was predominantly associated with *E. coli* followed by other implicated clinical conditions ranging from Wound sepsis, Flame burns, Chronic sinusitis, Retroviral disease on ART, Jaundice, Chronic liver disease, Dysuria, Lower respiratory tract infection, Cough, Multiple myeloma, Tuberculosis and Known Hbs with haemolytic crisis which constituted 7.14% of the mixed culture infection (**Table 3**).

The present study showed that the prevalence of *E. coli* was higher among the elderly 8 (57.14%) compared to young age patients 6 (42.85%). Overall prevalence of *E. coli* was equal for both sexes in the study area (**Table 4**). *E. coli* isolates from the female patients exhibited increased antibiotic resistance than isolates from male patients. The highest resistance was with nitrofurantoin, gentamycin, tetracycline and perfloracin followed by cotrimoxazole with perfloracin (**Table 5**). Least resistance was with ofloxacin. The highest susceptibility was with ceftriazone. Least susceptibility was with ceftriazone. Highest resistance was also with ofloxacin.

In the present study, 43.75% of the clinical samples which comprised *E. coli* showed polymicrobial association of isolates in many infections. This is in support of the findings of [11] on their investigation on urine culture. The present study showed the prevalence of drug resistant strains of *E. coli* being higher (92.85%) in comparison with previous studies carried out in Sudan. In this study, the prevalence of *E. coli* is relatively higher than those reported in neighboring countries such as in Egypt (87%) and in Ethiopia (74.6%) [11,12].

In this investigation, high resistance rates of *E. coli* was to certain first-line oral antimicrobial agents. These were amoxycillin (100%), cotrimoxazole (92.85%) and tetracycline (100%) (**Table 6**). These findings represent alarming increased rates in resistant *E. coli*. These results are comparable to findings in other studies [13,14].

Resistance to fluoroquinolones varies geographically and is an emerging problem in both developed and developing countries [15,16]. In this study, it is observed that the *E. coli* isolates showed relatively high resistance to ciprofloxacin and is in support of reports of [17], whose findings advocated the appropriate use of

Table 3 Cultural morphology of micro organism isolated from clinical sources.

| Sources of Isolation | Gender | Age | Diagnosis | Isolate code | Gram stain | Growth on Mac Conkey | Growth on EMB |
|----------------------|--------|-----|-----------------------------------|--------------|------------|----------------------|---------------|
| Wound biopsy | M | 60 | Wound sepsis | CLS1 | - | + | + |
| Wound biopsy | M | 44 | Flame burns | CLS2 | - | + | + |
| Antral washouts | F | 5 | Chronic sinusitis | CLS3 | - | + | + |
| Sputum | F | 2 | Retroviral on ART | CLS4 | - | + | + |
| Urine | M | 62 | Urinary tract infection | CLS5 | - | + | + |
| Ascitic fluid | F | 60 | Jaundice | CLS6 | - | + | + |
| Sputum | M | 65 | Chronic liver disease | CLS7 | - | + | + |
| Urine | M | 69 | Dysuria | CLS8 | - | + | + |
| Urine | F | 37 | Urinary tract infection | CLS9 | - | + | + |
| Sputum | M | 60 | Lower respiratory tract infection | CLS10 | - | + | + |
| Sputum | F | 13 | Cough | CLS11 | - | + | + |
| Tracheal aspirate | F | 41 | Multiple myeloma | CLS12 | - | + | + |
| Sputum | M | 26 | Tuberculosis | CLS13 | - | + | + |
| Blood | F | 38 | Known Hbs with haemolytic crisis | CLS14 | - | + | + |

Table 4 Biochemical characterisation of microorganism isolated from clinical source.

| Isolates | CLS1 | CLS2 | CLS3 | CLS4 | CLS5 | CLS6 | CLS7 | CLS8 | CLS9 | CLS10 | CLS11 | CLS12 | CLS13 | CLS14 |
|-------------------|----------------|----------------|-------------------|----------------|----------------|-------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Citrate | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Indole | + | + | - | + | + | + | + | + | + | + | + | + | + | + |
| Motility | + | + | - | + | + | + | + | + | + | + | + | + | + | + |
| Hydrogen sulphide | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| Catalase | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| MR | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| VP | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Urease | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| De-mannitol | + | + | + | + | + | - | + | + | + | + | + | + | + | + |
| Lactose | + | + | + | + | + | - | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | - | + | + | + | + | + | + | + | + |
| Possible organism | <i>E. coli</i> | <i>E. coli</i> | <i>Proteus sp</i> | <i>E. coli</i> | <i>E. coli</i> | <i>Proteus vulgaris</i> | <i>E. coli</i> |

Table 5 Frequency of antibiotic susceptibility of the *E. coli* isolated.

| Antibiotics with disc potency | Frequency % | | | |
|-------------------------------|-------------|--------------|-------------|-------|
| | Resistance | Intermediate | Susceptible | Total |
| Augmentin (30 µg) | - | - | - | - |
| Ceftriazone (30 µg) | 78.58 | 14.28 | 7.14 | 100 |
| Nitrofuranton (300 µg) | 100 | 0 | 0 | 100 |
| Gentamycin (10 µg) | 100 | 0 | 0 | 100 |
| Cotrimozazole (23.2 µg) | 92.86 | 7.14 | 0 | 100 |
| Ofloxacin (5 µg) | 57.15 | 14.28 | 28.57 | 100 |
| Ciprofloxacin (5 µg) | 92.86 | 7.14 | 0 | 100 |
| Amoxycillin (30 µg) | - | - | - | - |
| Tetracycline (30 µg) | 100 | 0 | 0 | 100 |
| Pefloxacin (5 µg) | 100 | 0 | 0 | 100 |

Table 6 Multiple Antibiotic Resistance Pattern of *E. coli* isolated from Clinical sources.

| Number of Antibiotic(s) | Antibiotic Resistance Pattern | Frequency | Total Number |
|-------------------------|---------------------------------|-------------|--------------|
| 1 | NIT | 4 (28.57%) | 4 (28.57%) |
| 2 | CRO/GEN | 1 (7.14%) | 2 (14.28%) |
| | NIT/GEN | 1 (7.14%) | |
| 5 | GEN/OFL/CPX/TET/PFX | 1 (7.14%) | 1 (7.14%) |
| 6 | NIT/GEN/COT/OFL/CPX/TET | 1 (7.14%) | 3 (21.42%) |
| | GEN/COT/OFL/CPX/TET/PFX | 1 (7.14%) | |
| | CRO/NIT/GEN/OFL/CPX/PFX | 1 (7.14%) | |
| 7 | NIT/GEN/COT/OFL/CPX/TET/PFX | 1 (7.14%) | 2(14.28%) |
| | CRO/GEN/COT/OFL/CPX/TET/PFX | 1 (7.14%) | |
| 8 | CRO/NIT/GEN/COT/OFL/CPX/TET/PFX | 1 (7.14%) | 1 (7.14%) |
| | TOTAL | 13 (92.83%) | 13(92.83%) |

KEY: CRO=CEFTRIAZONE; COT=COTRIMOZAZOLE; CPX=CIPROFLOXACIN; NIT=NITROFURANTON; OFL=OFLOXACIN; TET=TETRACYCLINE; GEN=GENTAMYCIN; AMX=AMOXYCILLIN

fluoroquinolones in humans.

Whilst the third-generation cephalosporin such as ceftriazone has been used to treat Gram-negative bacterial infections of various body sites [18], the current study showed high levels of resistance to ceftriazone (78.25%) in *E. coli*. This might be as a result of Extended Spectrum Beta-Lactamases (ESBL) in the strains [19].

Lower susceptibility rates were observed with ceftriazone (7.14%)

and ofloxacin (28.57%) while susceptibility to Cotrimozazole and Ciprofloxacin was 0%. Intermediate susceptibility was also observed with ceftriazone (14.28%), ciprofloxacin (7.14%) and cotrimozazole (7.14%). However, it was observed that a particular isolate, was resistant to all the antibiotics used. This may be attributed to the prevailing usage and abuse of drugs in the area under study. The resistance to these drugs may be attributed to indiscriminate drugs usage.

Recommendations

The judicious use of antibiotic by the health professional and efforts to control procurement and use of antibiotics officially in the locality will probably help to limit the increasing rate of drug

resistance in the pathogens. Also, it is imperative for optimal patient care that constant evaluation of antibiotic sensitivity pattern of pathogens for commonly used antimicrobial agents in a particular environment greatly maintained.

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