# MINIMUM INHIBITORY CONCENTRATIONS OF CEPHALOSPORIN ANTIBIOTICS FOR FECAL AND URINARY *ESCHERICHIA COLI* FROM UNIVERSITY STUDENTS IN KEFFI, NIGERIA

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## Abstract

*Minimum inhibitory concentration (MIC) provides in-vitro justification for confirming antibiotic resistance strains. This study evaluated the MICs of cephalexin, cefuroxime, ceftazidime and cefepime for Escherichia coli isolated from urine and stool of university students in Keffi. Eighty bacteria (thirty from urine, fifty from stool) were isolated and identified as E. coli from students by cultural, microscopic and biochemical methods. MICs were evaluated using macro-broth dilution method and breakpoint susceptibility interpreted as described by Clinical and Laboratory Standards Institute (CLSI) of the United States of America (USA). The isolates were also screened for carriage of extended spectrum beta-lactamase (ESBL). The antibiotic susceptibility level and MIC for 50% of the isolates ($MIC_{50}$) from urine was: cephalexin (0% and 45.8 µg/ml), cefuroxime (0% and 64.0 µg/ml), ceftazidime (20% and 18.4 µg/ml) and cefepime (47% and 13.8 µg/ml). Fecal isolates had susceptibility and $MIC_{50}$ as follows: cephalexin (8% and 34.2 µg/ml), cefuroxime (20% and 32.0 µg/ml), ceftazidime (32% and 8.0 µg/ml) and cefepime (46% and 4.8 µg/ml). With the exception of cefuroxime, the differences in $MIC_{50}$ values of each cephalosporin antibiotics tested for urine and stool isolates were insignificant. For both urinary and faecal isolates, MIC for 90% of the isolates ($MIC_{90}$) decreased in the order: cefepime < ceftazidime < cefuroxime and cephalexin. This study revealed high susceptibility pattern of urinary and faecal E. coli isolates from apparently healthy individual to a fourth generation cephalosporin cefepime. Furthermore, all isolates are potential carriers of extended spectrum beta-lactamases. Further investigation is required to confirm the isolates as ESBL carriers.*

**Key Words:** Minimum Inhibitory Concentration; Cephalosporin; Antibiotics; *Escherichia coli*

## INTRODUCTION

Antimicrobial agents remain the mainstay treatment for infections by *Escherichia coli*, a common gastro-intestinal tract bacterium found in the large intestine of humans and other warm-blooded animals (Campbell & Reece, 2002) which is responsible for many intestinal and extra-intestinal infections (Bailey *et al*., 2006). However, the continued usefulness of these agents is limited by the development of resistance mechanisms (Todar, 2007; Pitout & Laupland, 2008). Hence, the testing of the *in vitro* susceptibility of isolated bacteria to various antimicrobial agents is an important guide to antimicrobial therapy (Daivis & Dulbecco, 2000).

One important way to assess the antibiotic susceptibility of a bacterium is the determination of a "minimum inhibitory concentration" (MIC), the lowest concentration of an antimicrobial that will inhibit the visible growth of the tested organism. The performance of antimicrobial susceptibility testing for bacterial isolates is an important task in the clinical microbiology laboratory that helps to detect possible drug resistance in common pathogens and assures susceptibility to drugs of choice for particular infections.

Resistance in *E. coli* isolates to cephalosporins is increasingly reported worldwide (Akins *et al*., 2002; Forward *et al*., 2004; Johnson *et al*., 2007; Drawz & Bonomo, 2010; Ngwai *et al*., 2010; Thokar *et al*., 2010; Ngwai *et al*., 2011; Park *et al*., 2012; Talukdar *et al*., 2013). It is thus of great concern to monitor the susceptibility of cephalosporins. This study evaluates the susceptibility of *E. coli* isolated from feces

and urine of university students in Keffi to cephalosporin antibiotics by MIC measurement. The goal of testing is to assure the susceptibility to cephalosporins of choice in *E. coli* infections and to detect possible resistance to cephalosporins. MICs are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials (Andrews, 2001).

## MATERIALS AND METHODS

### Bacterial Isolates

A total of 80 *E. coli* isolates (30 fecal, 50 urinary) were used in this study. They were isolated and identified from stool or urine of students of Nasarawa State University Keffi using standard cultural, microscopical and biochemical procedures (Cheesbrough, 2000). Pink colonies on MacConkey agar (BIOTEC Laboratories Ltd., Ipswich, United Kingdom) that grew with greenish metallic sheen characteristics on eosin methylene blue agar (BIOTEC Laboratories Ltd., Ipswich, United Kingdom) and which were indole positive, methyl red positive, Voges-Proskauer negative and citrate negative were confirmed as *E. coli*. Bacteria were stored in the refrigerator at 4°C on nutrient agar (NA: Merck KGaA, Darmstadt, Germany) slants and reactivated by sub-culturing on MacConkey agar and used for the experiments.

### Antibiotics

The antibiotics used were cephalexin (Ranbaxy Laboratories Ltd, India), cefuroxime (Glaxo Smith-Kline, India), ceftazidime (Glaxo Smith-Kline, Italy) and cefepime (Bharat Parenterals Ltd., India). All antibiotics were purchased from the Pharmacy Department, Federal Medical Center, Keffi, Nasarawa State, Nigeria. The stock solutions were prepared in appropriate solvents in accordance with the method of Clinical and Laboratory Standards Institute (CLSI, 2012).

### Determination of Minimum Inhibitory Concentration (MIC)

The MICs of the antibiotics against the *E. coli* isolates and quality control strain (*E. coli* ATCC 25922) were determined in triplicate using the CLSI macro-broth dilution method (CLSI, 2012). An adjusted inoculum of the test organism was inoculated into Mueller-Hinton broth (MHB: BIOTEC Laboratories Ltd., Ipswich, United Kingdom) containing two-fold dilutions of an initial antibiotic solution so that each tube contained approximately 1 x $10^5$ colony-forming units (CFU). Results were observed and registered after 24-h incubation at 37°C. MIC was defined as the lowest concentration that inhibited visible growth. Cumulative frequency curves of the antibiotic MICs of isolates were plotted and MICs for 50% ($MIC_{50}$) and for 90% ($MIC_{90}$) of isolates were then generated from the plots.

### Statistical Analyses

$MIC_{50}$ and $MIC_{90}$ were analyzed by one way analysis of variance (ANOVA) using Smith Statistical Package (SSP), version 2.80 (by Gary Smith, Pomona College, Claremont, California).

## RESULTS

### MIC ranges of antibiotics

The MIC ranges of the antibiotics for the *Escherichia coli* isolates are as shown in Table 1.

### MIC for 50% and 90% of isolates

The MIC for 50% of isolates ($MIC_{50}$) and MIC for 90% of isolates ($MIC_{90}$) generated from the cumulative frequency curves in Figure 1 (urinary) and Figure 2 (fecal) are as shown in Table 2.

### Susceptibility of isolates

The susceptibilities of the isolates to cephalosporin antibiotics are as shown in Table 3. The urinary isolates are slightly more resistant to the cephalosporins than

**Table 1:** Minimum Inhibitory Concentration ranges of Cephalosporin antibiotics for the *Escherichia coli* isolates

| Antibiotics | MIC ranges (µg/ml) | |
|---|---|---|
| | Urinary | Fecal |
| Cephalexin | 32-512 | 8-128 |
| Cefuroxime | 32-512 | 8-128 |
| Ceftazidime | 4-128 | 8-128 |
| Cefepime | 2-128 | 2-128 |

**Table 2:** Minimum Inhibitory Concentration of cephalosporin antibiotics for 50% and 90% of the *Escherichia coli* isolates

| Antibiotics | $MIC_{50}$ (µg/ml) | | $MIC_{90}$ (µg/ml) | |
|---|---|---|---|---|
| | Urinary | Fecal | Urinary | Fecal |
| Cephalexin | 45.8 (≥ 4.0) | 34.2 (≥ 4.0) | 508.4 (≥ 4.0) | 118.0 (≥ 4.0) |
| Cefuroxime | 64.0 (≥ 4.0) | 32.0 (≥ 4.0) | 508.4 (≥ 4.0) | 116.6 (≥ 4.0) |
| Ceftazidime | 18.4 (≥ 16.0) | 8.0 (≤ 16.0) | 32.0 (≥ 16.0) | 61.0 (≥ 16.0) |
| Cefepime | 13.8 (≤ 16.0) | 4.8 (≤ 16.0) | 27.4 (≥ 16.0) | 20.0 (≥ 16.0) |

$MIC_{50}$ = Minimum Inhibitory Concentration for 50% of isolates; $MIC_{90}$ = Minimum Inhibitory Concentration for 90% of isolates.

the fecal isolates. Both isolate types were similarly susceptible to cefepime, a fourth generation cephalosporin antibiotic.

**Statistical Analyses**

Antibiotic $MIC_{50}$ and $MIC_{90}$ for urine isolates were compared with those for fecal isolates. Significance of differences between the values were determined at p=0.05 (5% probability) as shown in Table 4. The statistical analyses indicated that the antibiotics have insignificant p-values; while the comparison of the sources of the isolates was be significant.

**DISCUSSION**

The testing of the *in vitro* susceptibility of isolated bacteria to various antimicrobial agents is an important guide to therapy (Daivis & Dulbecco, 2000). This is because the susceptibility of bacterial strains to a given antibiotic can grossly affect the *in vivo* efficacy of that antibiotic, among other factors like age, immune status of patient, existing disorder and route of antibiotic administration (Mandell, 2002). The determination of MIC is one important way to assess the antibiotic susceptibility of a bacterium. Minimum inhibitory concentration values are used in diagnostic laboratories, mainly to confirm resistance of strains of bacteria (Andrew, 2001). This study evaluated the minimum inhibitory concentration of cephalosporin antibiotics against *E. coli* from fecal and urinary sources among university students in Keffi.

With MIC values obtained for some of the isolates above the CLSI resistance breakpoint of ≥4 µg/ml (cephalexin and cefuroxime), ≥16 µg/ml (ceftazidime) and ≥32 µg/ml cefepime) (CLSI, 2010), such isolates are said to be resistant to the cephalosporin antibiotics tested. Previous studies on *E. coli* elsewhere have reported cephalosporin resistance (Diekema *et al*., 2000; Feng *et al*., 2002; Ehinmidu, 2003; Ngwai et al., 2005; Zing *et al.*, 2006; Ngwai *et al*., 2010; Ngwai *et al*., 2011; Park *et al*., 2012; Talukdar *et al*., 2013).

Since MIC values of ceftazidime obtained for 50% and 90% of isolates at ≥2 µg/ml, these isolates are potential carriers of extended spectrum beta-lactamases (ESBL) since each *E.*

*coli*, *Klebsiella pneumoniae* or *K. oxytoca* isolate is considered a potential ESBL producer if MIC test results show ≥2 μg/ml for at least one of the extended-spectrum cephalosporins (cefpodoxime, ceftazidime, ceftriaxone or cefotaxime) and monobactams (e.g. aztreonam) (NCCLS, 1999). The possibility of our isolates being ESBL carriers is justified by the fact that studies using cefpodoxime or ceftazidime show the highest sensitivity for ESBL detection (Ho *et al*., 2000; MacKenzie *et al*., 2002). Many clinical and non-clinical *E. coli* isolates are known to produce extended spectrum beta-lactamases (ESBL) which are plasmid borne (Paterson & Bonomo, 2005; Pitout & Laupland, 2008; Isendahl *et al*., 2012; Al-Mayahie, 2013).

The generally higher susceptibility observed for the *E. coli* isolates to cefepime is not new (Iqbal *et al*., 2002); and should not be taken for granted to suggest clinical usefulness in view of CLSI's recommendation that all cephalosporin susceptibility results be reported as "resistant" when an isolate is determined to produce ESBL (CLSI, 2009).

**Table 3:** Susceptibility of *Escherichia coli* isolates to cephalosporin antibiotics

| **Antibiotics** | **Number (%) Susceptibility** | |
|---|---|---|
| | **Urinary (n = 30)** | **Fecal (n = 50)** |
| Cephalexin | 0 (0) | 4 (8) |
| Cefuroxime | 0 (0) | 10 (20) |
| Ceftazidime | 6 (20) | 16 (32) |
| Cefepime | 14 (47) | 23 (46) |

**Table 4:** Statistical analyses of Minimum Inhibitory Concentration of Cephalosporin antibiotics for 50% and 90% of *Escherichia coli* isolates

| **Statistics** | **Isolate Type** | **P value** | **Remarks (at P = 0.05)** |
|---|---|---|---|
| $MIC_{50}$ Cephalexin | Urine vs Stool | 0.4226 | Insignificant |
| $MIC_{50}$ Cefuroxime | Urine vs Stool | 0.0073 | Significant |
| $MIC_{50}$ Ceftazidime | Urine vs Stool | 0.1703 | Insignificant |
| $MIC_{50}$ Cefepime | Urine vs Stool | 0.5206 | Insignificant |
| $MIC_{90}$ Cephalexin | Urine vs Stool | 0.0029 | Significant |
| $MIC_{90}$ Cefuroxime | Urine vs Stool | 0.0029 | Significant |
| $MIC_{90}$ Ceftazidime | Urine vs Stool | 0.1358 | Insignificant |
| $MIC_{90}$ Cefepime | Urine vs Stool | 0.0002 | Significant |

$MIC_{50}$ = Minimum Inhibitory Concentration for 50% of isolates; $MIC_{90}$ = Minimum Inhibitory Concentration for 90% of isolates.

The result from this study provides possible evidence for higher susceptibility, to the fourth generation cephalosporin cefepime, by *E. coli* isolated from university students in Keffi decreasing in the order: cefepime < ceftazidime < cefuroxime < cephalexin. Furthermore, all isolates are potential carriers of extended spectrum beta-lactamases. Further investigation is required to confirm the isolates as ESBL carriers.

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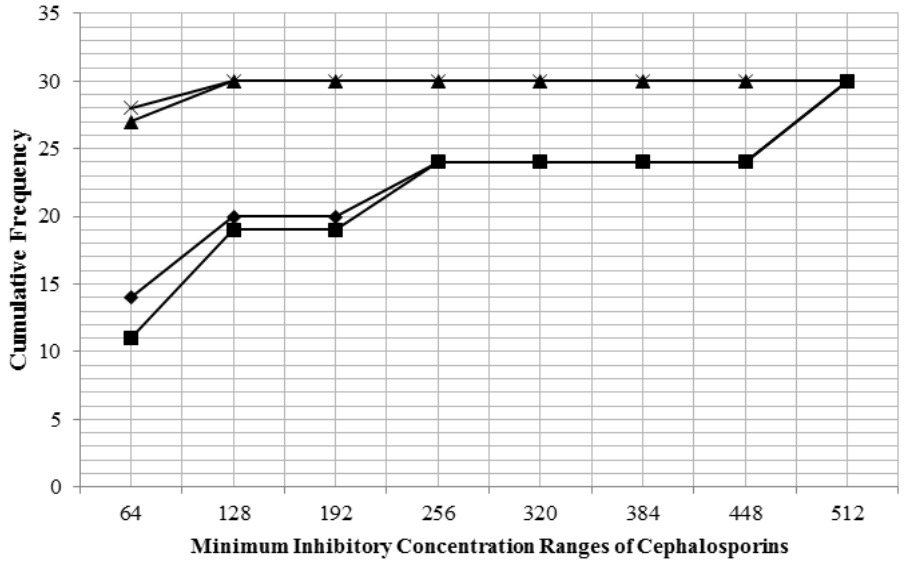


**Figure 1:** Cumulative Frequency Curves of Cephalosporin MICs for urinary isolates of *Escherichia coli* (◆Cephalexin, ■Cefuroxime, ▲Ceftazidime, X Cefepime).

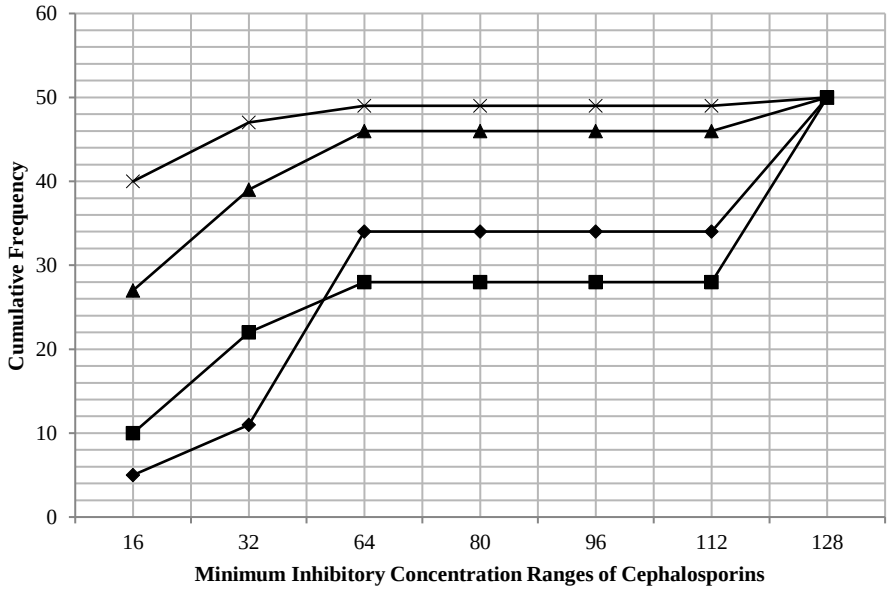


**Figure 2:** Cumulative Frequency Curves of Cephalosporin MICs for fecal isolates of *Escherichia coli* (Cephalexin◆ , Cefuroxime ■, Ceftazidime ▲, Cefepime X ).

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