Antimicrobial Susceptibility Profile of Extended Spectrum β-Lactamase (ESBL) Producing Escherichia coli from Various Clinical Samples

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ABSTRACT

BACKGROUND: Extended spectrum β-lactamase (ESBL) producing Escherichia coli has tremendously increased worldwide and it is one of the most common causes of morbidity and mortality associated with hospital-acquired infections. This could be attributed to association of multi drug resistance in ESBL producing isolates. The present study was aimed to determine the antimicrobial sensitivity profile of ESBL producing E. coli isolates from various clinical samples.

MATERIALS AND METHODS: Clinical samples, which consist of pus, urine, blood, cerebrospinal fluid (CSF), stool, sputum, swabs, and different body fluids, are included in the study. Samples were processed and identified as per routine laboratory protocol. ESBL screening and confirmation along with antimicrobial susceptibility test was done according to the Clinical Laboratory Standards Institute (CLSI) guidelines.

RESULTS: Out of 180 third generation cephalosporins resistant E. coli, 100 (55.55%) isolates were ESBL producers showing a greater degree of resistance to antibiotics.

CONCLUSION: The prevalence of ESBL is increasing day by day in nearly every center of different countries and necessary steps to prevent the spread and emergence of resistance should be taken.

KEYWORDS: ESBL, E. coli, ESBL producing E. coli, DDS

Resistant bacteria are emerging world wide as a threat to favorable outcomes of treatment of common infections in community and hospital settings. Urinary tract, gastrointestinal, and pyogenic infections are the common hospital-acquired infections caused by members of Enterobacteriaceae. Among Enterobacteriaceae, Escherichia coli has been the most commonly isolated species. E. coli are very well known to exhibit multidrug resistance. Prolonged antibiotic exposure, overstayed in hospitals, severe illness, unprecedented use of third generation cephalosporin, and increased use of intravenous devices or catheters are important risk factors for infection with multidrug resistant E. coli. β-lactamase production is perhaps the single most important mechanism of resistance to penicillins and cephalosporins. E. coli possess a naturally occurring chromosomally mediated β-lactamase or plasmid mediated β-lactamases. These enzymes are thought to have evolved from penicillin binding proteins. This development was likely to be because of selective pressure exerted by β-lactam producing soil organisms found in the environment. In early 1960s, TEM-1 was the first plasmid mediated β-lactamase described in Gram-negative organisms. Another common plasmid mediated β-lactamase is SHV – 1.

Extended spectrum β-lactamases (ESBLs), enzymes that show increased hydrolysis of oximino-β-lactams, which include...
isolates recovered from clinical samples including biochemical reactions: µ
Colony morphology µ
µ
µ
Biochemical reactions were performed by inoculating the organism was labeled as sensitive, resistant, or intermediate as per CLSI 2012 guidelines (Table 1). 9

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines. 9 Commercially available antibiotic disks (HiMedia Labs, India) were used for antimicrobial susceptibility testing. The following antibiotic disks were used, ampicillin (10 µg), pipercillin (100 µg), pipercillin-tazobactam (100/10 µg), amoxicillin/clavulanic acid (20/10 µg), cefoperazone/sulbactam (75/10 µg), ceftazidime/clavulanate (30/10 µg), cefoperazone (75 µg), cefoxitin (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftizoxime (30 µg), aztreonam (30 µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin (30 µg), olofoxacin (5 µg), norfloxacin (10 µg), and nitrofurantoin (300 µg).

Procedure. Inoculum of 0.5 McFarland standards turbidity was prepared in a nutrient broth from isolated colony of E. coli selected from 18–24 hour agar plates. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level and inoculated on the dried surface of a Mueller-Hinton agar (MHA) plate by streaking the swab over it. For even distribution of inoculum, the swab was streaked two more times at 60°C over the agar surface. After 3–5 minutes, antibiotic disks were applied and pressed down to ensure complete contact with agar surface. The discs were distributed evenly to ensure a minimum distance of 24 mm from center to center. The plates are then inverted and incubated aerobically at 37°C within 15 minutes of disc application.

Interpretation. Diameter of zone of inhibitions were measured and recorded in millimeters with the help of sliding calipers and organism was labeled as sensitive, resistant, or intermediate as per CLSI 2012 guidelines (Table 1). 9
Table 1. Zone diameter interpretative criteria for *E. coli*.

| ANTIBIOTIC DISC                              | SENSITIVE | INTERMEDIATE | RESISTANT |
|---------------------------------------------|-----------|--------------|-----------|
| Penicillins                                 |           |              |           |
| Ampicillin                                  | =17       | 14–16        | =13       |
| Piperacillin                                | ≥21       | 18–20        | =17       |
| β-lactam/β-lactamase inhibitors combinations |           |              |           |
| Piperacillin/Tazobactam                     | ≥21       | 18–20        | =17       |
| Amoxicillin/Claulanic acid                  | ≥18       | 14–17        | =13       |
| Cefoperazone/Sulbactam*                     | ≥21       | 16–20        | =15       |
| Ceftazidime/Clavulanate*                    | ≥21       | 18–20        | =17       |
| Cepheems (Parenteral)                       |           |              |           |
| Cefoperazone                                | ≥21       | 16–20        | =15       |
| Cefoxitin                                   | ≥18       | 15–17        | =14       |
| Ceftazidime                                 | ≥21       | 18–20        | =17       |
| Cefotaxime                                  | ≥26       | 23–25        | =22       |
| Ceftriazone                                 | ≥23       | 20–22        | =19       |
| Ceftepime                                   | ≥18       | 15–17        | =14       |
| Monobactam                                  |           |              |           |
| Aztreonam                                   | ≥21       | 18–20        | =17       |
| Carbapenem                                  |           |              |           |
| Imipenem                                    | ≥23       | 20–22        | =19       |
| Aminoglycosides                             |           |              |           |
| Gentamicin                                  | ≥15       | 13–14        | =12       |
| Amikacin                                    | ≥17       | 15–16        | =14       |
| Fluoroquinolones                            |           |              |           |
| Ciprofloxacin                               | ≥21       | 16–20        | =15       |
| Ofloxacin                                   | ≥16       | 13–15        | =12       |
| Norfloxacin                                 | ≥17       | 13–16        | =12       |
| Nitrofuran                                  |           |              |           |
| Nitrofurantoin                              | ≥17       | 15–16        | =14       |

*Cefoperazone breakpoints were used for Cefoperazone/Sulbactam and Ceftazidime breakpoints were used for Ceftazidime/Clavulanate, as no zone diameter interpretative criteria are currently provided by CLSI for these drug combination.

The quality control of antibiotic sensitivity was done using *E. coli* ATCC 25922 and *E. coli* ATCC 35218 (for β-lactam/β-lactamase inhibitor combination).

ESBL detection methods. *E. coli* were first screened for ESBL production by phenotypic method and then phenotypic confirmatory test was done as per CLSI guidelines 2012.9

(a) Phenotypic screening of ESBL. CLSI 2012 has recommended the use of any of the following antibiotic discs for screening of ESBL producers. Antibiotic disks of ceftazidime, aztreonam, cefotaxime, and ceftriazone were used. More than one of these agents was used for screening to improve the sensitivity of ESBL detection, as CLSI has recommended the method only in 2012 guidelines.

**Procedure.** Inoculum with turbidity equivalent to 0.5 McFarland standards was prepared from colonies on agar plates. MHA plates were inoculated by lawn culture method using a sterile cotton swab. With a sterile forceps ceftazidime, cefotaxime, ceftriazone, and aztreonam disks were placed on the MHA plate and the plate was incubated at 35°C for 18–24 hours.

**Interpretation of results.** Zones given below, against respective antibiotic indicate potential ESBL producer. If any strain was suspected as ESBL producer then phenotypic confirmatory tests were done.

- Ceftazidime ≤ 22 mm or
- Aztreonam ≤ 27 mm or
- Ceftriazone ≤ 25 mm or
- Cefotaxime ≤ 27 mm

(b) Phenotypic confirmatory methods. Confirmatory test was done by two methods.
In this study, samples were collected from different wards/OPDs. All the 180 isolates of *E. coli* were tested by Kirby–Bauer disk diffusion method for antimicrobial susceptibility pattern. Highest susceptibility was found to imipenem (100%) followed by piperacillin/tazobactam (87.22%), cefoperazone/sulbactam (76.67%), amoxicillin/clavulanic acid (75.55%), and cefazidime/clavulanate (66.11%). *E. coli* were resistant to most of the drugs used as first line drugs. A low susceptibility was observed with third generation cephalosporin and β-lactam antibiotic was further decreased as compared to non-ESBL producing *E. coli* (Table 4). Susceptibility to ESBL inhibitor combination drugs was almost the same as compared to non-ESBL producing *E. coli*.

### Table 2. Distribution of *E. coli* on the basis of source.

| SPECIMEN | IN-PATIENTS | OUT-PATIENTS |
|----------|-------------|--------------|
| Urine    | 41          | 34           |
| Pus      | 14          | 11           |
| Wound    | 8           | 12           |
| Aspirate | 14          | 6            |
| Blood    | 14          | 1            |
| Ear swab | 11          | 4            |
| Stool    | 2           | 5            |
| Sputum   | 1           | 2            |
| Total    | 105         | 75           |

**Discussion**

The discovery and development of antibiotics was undoubtedly one of the greatest advances of modern medicine. Unfortunately the emergence of antibiotic resistance bacteria is threatening the effectiveness of many antimicrobial agents. This has increased the hospital stay of the patients, which in turn causes economic burden. In the present study, an attempt was made to understand the prevalence of ESBL producing *E. coli*. The present study was based on laboratory findings and includes the patients attending the out-patient and in-patient departments of Sharda hospital during a period from September 2010 to March 2012. On screening with third generation cephalosporin, a total of 180 *E. coli* clinical isolates were selected and studied for their antimicrobial susceptibility and β-lactamase productions such as ESBL.

In this study, samples were collected from different wards/OPDs. All the 180 isolates of *E. coli* were tested by Kirby–Bauer disk diffusion method for antimicrobial susceptibility pattern. Highest susceptibility was found to imipenem (100%) followed by piperacillin/tazobactam (87.22%), cefoperazone/sulbactam (76.67%), amoxicillin/clavulanic acid (75.55%), and cefazidime/clavulanate (66.11%). *E. coli* were resistant to most of the drugs used as first line drugs. A low susceptibility was observed with third generation cephalosporin (cefotaxime, ceftazidime, and ceftriaxone) (31.11 and 35.55, 38.33%, respectively), cephamycin (cefotixin) (31.11%), monobactam (aztreonam) (31.11%), piperacillin (33.33%), cefazidime (27.77%), and cefepime (35.55%). When the susceptibility of *E. coli* isolated from pus, urine, and blood was...
Table 3. Antimicrobial susceptibility pattern of *E. coli*.

| ANTIBIOTICS                        | URINE          | PUS            | BLOOD          | TOTAL SAMPLE  |
|------------------------------------|----------------|----------------|----------------|---------------|
|                                    | n = 75 SENSITIVE | n = 25 SENSITIVE | n = 15 SENSITIVE | n = 180 SENSITIVE |
|                                    | NO. (%)        | NO. (%)        | NO. (%)        | NO. (%)       |
| Penicillins                        |                |                |                |               |
| Ampicillin                         | 25 (33.33%)    | 7 (28%)        | 5 (33.33%)     | 54 (30%)      |
| Piperacillin                       | 32 (42.66%)    | 10 (40%)       | 9 (60%)        | 60 (33.33%)   |
| β-lactam/β-lactamase inhibitor combinations |            |                |                |               |
| Piperacillin/Tazobactam            | 68 (90.66%)    | 2 (84%)        | 13 (86.66%)    | 157 (87.22%)  |
| Amoxicillin/Clavulanic acid        | 60 (80%)       | 18 (72%)       | 11 (73.33%)    | 136 (75.55%)  |
| Cefoperazone/Subactam              | 62 (82.66%)    | 20 (80%)       | 13 (86.66%)    | 138 (76.67%)  |
| Ceftazidime/Clavulanate            | 57 (76%)       | 18 (72%)       | 11 (73.33%)    | 119 (66.11%)  |
| Cepham (Parenteral)                |                |                |                |               |
| Cefoperazone                       | 23 (30.66%)    | 7 (28%)        | 8 (53.33%)     | 50 (27.77%)   |
| Cefoxitin                          | 23 (30.66%)    | 7 (28%)        | 5 (33.33%)     | 56 (31.11%)   |
| Ceftazidime                        | 25 (33.33%)    | 8 (32%)        | 5 (33.33%)     | 64 (35.55%)   |
| Cefotaxime                         | 21 (28%)       | 7 (28%)        | 6 (40%)        | 56 (31.11%)   |
| Ceftriazone                        | 25 (33.33%)    | 8 (32%)        | 6 (40%)        | 69 (38.33%)   |
| Cefepime                           | 27 (36%)       | 7 (28%)        | 4 (26.66%)     | 64 (35.55%)   |
| Monobactam                         |                |                |                |               |
| Aztreonam                          | 28 (37.33%)    | 7 (28%)        | 4 (26.66%)     | 56 (31.11%)   |
| Carabapenem                        |                |                |                |               |
| Imipenem                           | 75 (100%)      | 25 (100%)      | 15 (100%)      | 180 (100%)    |
| Aminoglycosides                    |                |                |                |               |
| Gentamicin                         | 56 (74.66%)    | 12 (48%)       | 9 (60%)        | 135 (75%)     |
| Amikacin                           | 60 (80%)       | 15 (60%)       | 10 (66.66%)    | 144 (80%)     |
| Flouroquinolones                   |                |                |                |               |
| Ciprofloxacin                      | 41 (54.66%)    | 13 (52%)       | 10 (66.66%)    | 84 (46.66%)   |
| Ofloxacin                          | 40 (53.33%)    | 13 (52%)       | 8 (53.33%)     | 97 (53.88%)   |
| Norfloxacin                        | 49 (65.33%)    |                |                |               |
| Nitrofuran                         |                |                |                |               |
| Nitrofurantoin                     | 50 (66.66%)    |                |                |               |

Table 4. Distribution of the various sources of ESBL producing *E. coli*.

| SPECIMEN    | NO. OF ISOLATES | PERCENTAGE |
|-------------|-----------------|------------|
| Blood       | 10              | 66.67%     |
| Aspirate    | 13              | 65.00%     |
| Stool       | 4               | 57.14%     |
| Wound       | 11              | 55.00%     |
| Urine       | 41              | 54.67%     |
| Pus         | 13              | 52.00%     |
| Ear         | 7               | 46.67%     |
| Sputum      | 1               | 33.33%     |
| Total       | 100             |            |

studied separately, it was found that the susceptibility pattern to the mentioned drugs remain the same with slight variation in the above quoted values.

Akram et al and Padmini et al also reported 100% susceptibility of urinary isolates of *E. coli* to imipenem.\(^{10,11}\) Menon et al in their study reported almost similar results of susceptibility for imipenem, piperacillin/tazobactam, cefoperazone/subactam, and ceftazidime/clavulanate with slight variation.\(^12\) Similar susceptibility patterns were also observed in studies conducted outside India. Kibret et al showed a high resistance to amoxicillin (86.0%) and tetracycline (72.6%) but a significantly high degree of susceptibility to nitrofurantoin (96.4%), norfloxacin (90.6%), and gentamicin (79.6%).\(^13\) Bamford et al demonstrated a significant decline in susceptibility to
Table 5. Distribution of ESBL producing \textit{E. coli} in in-patients and out-patients sample.

|                      | IN-PATIENTS | OUT-PATIENTS |
|----------------------|-------------|--------------|
| ESBL producers       | 64 (60.95%) | 36 (48%)     |
| Non-ESBL producers   | 41 (39.04%) | 39 (52%)     |
| Total                | 105         | 75           |

β-lactam antibiotics and fluoroquinolones, while susceptibility to amikacin and gentamicin remained significantly high.\cite{14}

In the present study, out of 180 \textit{E. coli}, 55.55% were ESBL producers by phenotypic confirmatory methods. The prevalence of ESBL producing \textit{E. coli} varies from country to country and from center to center. In the United States, ESBL producing \textit{E. coli} ranges from 0 to 25% with the average being around 3%.\cite{15} In Japan, the prevalence of ESBL producing \textit{E. coli} is <0.1%.\cite{16} In Asia, the percentage of ESBL production in \textit{E. coli} is 4.8, 8.5, and up to 12% in Korea, Taiwan, and Hong Kong, respectively.\cite{17-19} In India, the percentage of ESBL producers ranges from 22 to 75%.\cite{20-23}

ESBL producing \textit{E. coli} were isolated from all sites of the body from which samples were obtained namely, blood, urine, sputum, wound, pus, ear, stool, and aspirates. More than 50% of the isolates from blood, aspirate, stool, wound, urine, and pus were ESBL producers with blood accounting for the highest incidence of ESBL producers. This observation is of serious concern because of the severity of blood stream infections.

In our study, prevalence of ESBL among in-patients and out-patients was 60.95 and 48%, respectively. Although the prevalence of ESBL in out-patients is less than in-patients, it is common in communities. This is because ESBL producing \textit{E. coli} isolates were wide spread among both in-patients and

Table 6. Antimicrobial susceptibility pattern of ESBL producing \textit{E. coli} in urine and blood.

| ANTIBIOTICS                                    | URINE (n = 41) | BLOOD (n = 10) |
|-----------------------------------------------|---------------|--------------|
|                                               | SENSITIVE (%) | SENSITIVE (%)|
| Penicillins                                   |               |              |
| Ampicillin                                    | 3 (7.31%)     | 1 (10%)      |
| Piperacillin                                  | 8 (19.51%)    | 3 (30%)      |
| β-lactam/β-lactamase inhibitors combinations  |               |              |
| Piperacillin/Tazobactam                       | 33 (80.48%)   | 7 (70%)      |
| Amoxicillin/Clavulanic acid                   | 28 (68.29%)   | 7 (70%)      |
| Cefoperazone/Sulbactam                       | 29 (70.73%)   | 8 (80%)      |
| Ceftazidime/Clavulanate                      | 29 (70.73%)   | 7 (70%)      |
| Cephems (Parenteral)                         |               |              |
| Cefoperazone                                  | 8 (19.51%)    | 2 (20%)      |
| Cefotaxime                                    | 7 (17.07%)    | 2 (20%)      |
| Ceftriaxone                                   | 5 (12.19%)    | 2 (20%)      |
| Cefepime                                     | 7 (17.07%)    | 3 (30%)      |
| Monobactam                                    |               |              |
| Aztreonam                                     | 7 (17.07%)    | 1 (10%)      |
| Carbapenem                                    |               |              |
| Imipenem                                      | 41 (100%)     | 10 (100%)    |
| Aminoglycosides                               |               |              |
| Gentamicin                                    | 28 (68.29%)   | 6 (60%)      |
| Amikacin                                      | 30 (73.17%)   | 6 (60%)      |
| Fluoroquinolones                              |               |              |
| Ciprofloxacin                                 | 12 (29.26%)   | 4 (40%)      |
| Ofloxacin                                     | 15 (36.58%)   | 5 (50%)      |
| Norfloxacin                                   | 24 (58.53%)   |              |
| Nitrofuran                                    |               |              |
| Nitrofurantoin                                | 24 (58.53%)   |              |
out-patients. This observation therefore confirms the assertion by Pitout et al that ESBL producers are indeed as much a problem in the communities as in the hospitals.24

ESBL producers may have spread through communities, especially those with poor hygienic and sanitation conditions, through fecal contamination of soil and water, since most patients with ESBL producers may have had their gastrointestinal tracts colonized for a long period of time by these organisms as was reported by Paterson and Bonomo (2005).5

In vitro susceptibility studies of ESBL producing E. coli isolated from blood and urine showed that drug resistance was higher in ESBL producers than non-ESBL producers. Analysis of antimicrobial susceptibility pattern of ESBL producing E. coli isolates demonstrated high susceptibility rates to imipenem (100%), β-lactam/β-lactamase inhibitor combination drugs such as piperacillin/tazobactam (80.48, 70%), cefoperazone/sulbactam (70.73, 80%), ceftazidime/clavulanate (70.73, 70%), amoxicillin/clavulanic acid (68.29, 70%), and aminglycosides such as amikacin (73.17, 60%) and gentamicin (68.29, 60%) from urine and blood, respectively. High resistance rates were observed to penicillins such as ampicillin and piperacillin, third and fourth generation cephalosporins and fluoroquinolones. Norfloxacin and nitrofurantoin have good susceptibility against ESBL producing E. coli isolated from urine. So these drugs are recommended for the treatment of infections caused by ESBL producing E. coli. The carbapenems, on the other hand, should be used to treat only serious or life threatening infections in order to minimize cases of carbapenem resistance, though rare.5 In a study conducted by Ankur et al on clinical isolates of ESBL producing E. coli, resistance found to amikacin was 14.7%, gentamicin 66.7%, trimethoprim/sulfamethoxazole 79.1%, and ciprofloxacin 93.8%.25 Maina et al documented a higher proportion of isolates resistant to ciprofloxacin, levofloxacin, and tetracycline, and approximately 100% sensitivity to carbapenems.26 Al-Zarouni et al also demonstrated high resistance rates to fluoroquinolones and cephalosporins and higher susceptibility rates to carbapenems and amikacin.27

The ESBL producing E. coli are a cause of concern to the microbiologist as well as to the clinicians, particularly the multi drug resistant strains. Correct choice of antimicrobial agents according to the sensitivity profile is essential for appropriate empirical treatment. In the present study, no resistance was shown to carbapenem (imipenem). So, we suggest the use of carbapenem as the drug of choice for ESBL producers causing life threatening infections. However, antimicrobial susceptibility testing should be performed for each strain before prescribing antibiotics. The carbapenem should be used as a reserve drug only in cases of multi drug resistant strains. Carbapenem resistance in E. coli is only beginning to emerge as a clinical issue, yet the attention it has already received serves to underscore the seriousness of the problem. If past experience with multi drug resistant organisms is any indicator, the problem of carbapenem resistant E. coli will only grow in future.

Author Contributions
Conceived and designed the experiments: DK, MRA, YC. Analyzed the data: DK, AKS, MRA. Wrote the first draft of the manuscript: AKS. Contributed to the writing of the manuscript: DK, AKS, MRA. Agree with manuscript results and conclusions: DK, AKS, MRA, YC. Jointly developed the structure and arguments for the paper: DK, AKS, MRA. Made critical revisions and approved final version: DK, AKS. All authors reviewed and approved of the final manuscript.

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DISCLOSURES AND ETHICS
As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.
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