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Infectious Diseases: Research and Treatment

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

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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, overstay 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*.[1]

β-lactamase production is perhaps the single most important mechanism of resistance to penicillins and cephalosporins.[1] *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.[2]

Extended spectrum β-lactamases (ESBLs), enzymes that show increased hydrolysis of oxyimino-β-lactams, which include

cefotaxime, ceftriaxone, ceftazidime, and aztreonam, have been reported increasingly in recent years.[3] They belong to Ambler molecular class A and Bush–Jacoby functional group 2be.[4] These enzymes have been identified in large numbers from different regions and are significantly detected in various *E. coli* strains. They have also been found in other members of Enterobacteriaceae such as *Klebsiella spp*, *Citrobacter spp*, *Enterobacter spp*, *Proteus spp* and non-lactose fermenters like *Pseudomonas aeruginosa*.[2] Today over 200 different ESBLs have been described.[5] Major outbreak involving these resistant organisms has been reported all over the world in many members of the Enterobacteriaceae and *Pseudomonas spp*, resulting in limitation of therapeutic options.

ESBL producing strains are probably more prevalent than is currently recognized because they often remain undetected by routine susceptibility testing methods.[6] ESBL strains have been associated with resistance to other non β-lactam antibiotics like the aminoglycosides and chloramphenicol. Another property of these ESBL strains is that they might show a false sensitive zone of inhibition in the Kirby–Bauer disk diffusion method.[7]

Current knowledge of prevalence of ESBL production by commonly isolated organism such as *E. coli* is necessary to understand the disease burden and to take necessary action to prevent the spread. Therefore the present study was conducted with an objective to find out the prevalence of ESBL producing *E. coli* and its antimicrobial resistance profile to formulate effective antibiotic strategy and plan a proper hospital infection control strategy to prevent the spread of these strains.

Materials and Methods

E. coli isolates recovered from clinical samples including pus, urine, blood, cerebrospinal fluid (CSF), stool, sputum, ear swab, and different body fluids received in the bacteriology laboratory in the department of microbiology, School of Medical Sciences & Research, Greater Noida from inpatient and out-patient departments of Sharda Hospital during the period from September 2010 to March 2012 were included in the study. Ethical approval was obtained from Ethical Committee, School of Medical Sciences & Research, Greater Noida, India.

Isolation and identification. Urine samples collected in universal container, approximately 50 mL in amount, were inoculated using an inoculating loop of 10 µL volume calibration on cysteine lactose electrolyte deficient (CLED) agar plates. Other liquid specimens such as CSF, sputum, stool, and different body fluids collected in sufficient amount were inoculated on the blood agar plates and MacConkey agar plates using an inoculating loop. Blood samples collected in brain heart infusion (BHI) broth in a ratio of 1:5 (blood/broth) were first incubated overnight at 37°C and then subcultured on blood agar plates and MacConkey agar plates. Swabs were first rolled on blood agar plates and MacConkey agar plates and then streaked using an inoculating loop. All inoculated media were incubated aerobically overnight at 37°C. On the basis of colony morphology, gram staining, motility, and biochemical reactions, the organisms were identified as *E. coli*. Biochemical reactions were performed by inoculating the colony in a nutrient broth at 37°C for 2–3 hours. Following criteria was used for identification of *E. coli*.[8]

- **Colony morphology**: small 2–3 mm diameter, circular in shape, regular margin, flat, smooth, lactose fermenting, and translucent.
- **Gram staining:** Gram-negative rods, 1–3 × 0.3–0.5 µm in size, uniformly stained with no particular arrangement, non-sporing, and non-capsulated.
- **Motility:** motile bacteria in hanging drop preparation.
- **Biochemical reactions:** Oxidase negative, catalase positive, O/F test showed glucose fermentation, motility and gas production, reduces nitrates to nitrites, indole positive, methyl red positive, Voges–Proskauer negative, citrate not utilized, lactose fermenter, triple sugar iron agar showed both butt and slant yellow with gas production, lysine decarboxylase test positive.

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), piperacillin (100 µg), piperacillin-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), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), ofloxacin (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 discs 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/Clavulanic acid	≥18	14–17	≤13
Cefoperazone/Sulbactam*	≥21	16–20	≤15
Ceftazidime/Clavulanate*	≥21	18–20	≤17
Cephems (Parenteral)			
Cefoperazone	≥21	16–20	≤15
Cefoxitin	≥18	15–17	≤14
Ceftazidime	≥21	18–20	≤17
Cefotaxime	≥26	23–25	≤22
Ceftriaxone	≥23	20–22	≤19
Cefepime	≥18	15–17	≤14
Monobactam			
Aztreonam	≥21	18–20	≤17
Carbapenem			
Imipenen	≥23	20–22	≤19
Aminoglycosides			
Gentamicin	≥15	13–14	≤12
Amikacin	≥17	15–16	≤14
Flouroquinolones			
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 to 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 ceftriaxone 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, ceftriaxone, 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
- Ceftriaxone ≤ 25 mm or
- Cefotaxime ≤ 27 mm

(b) Phenotypic confirmatory methods. Confirmatory test was done by two methods.

i. Double disk diffusion test. Double disk approximation or double disk synergy (DDS) is a disk diffusion test in which 30 μg antibiotic disks of ceftazidime, ceftriaxone, cefotaxime, and aztreonam are placed on the lawn culture plate of *E. coli* on MHA, 30 mm (center to center) from the amoxicillin/clavulanic acid (20/10 μg) disk. This plate was incubated aerobically overnight at 37°C and examined for an extension of the edge of zone of inhibition of antibiotic disks toward the disk containing clavulanate. It is interpreted as synergy, indicating the presence of an ESBL.

ii. Cephalosporin/clavulanate combination disks. Cefotaxime (30 μg) or ceftazidime disks with (30 μg) or without clavulanate (10 μg) was used for phenotypic confirmation of the presence of ESBL as recommended by CLSI 2012 guidelines.[9] A lawn culture of *E. coli* was made on the MHA plate and disks were placed at an appropriate distance from each other and incubated aerobically overnight at 37°C. A difference in zone of inhibition of ≥5 mm of either of cephalosporin disks and their clavulanate containing disks indicates production of ESBL.

Statistical analysis. Chi-square test is used for statistical analysis of the data. A '*P* value' less than 0.05 was considered statistically significant.

Results

Among all the isolates, only 180 non-duplicate isolates of *E. coli* that showed resistance to third generation cephalosporins were included in this study without applying any selection criteria for the patients. Distribution of isolates on the basis of the source is documented in Table 2. *E. coli* was isolated in the highest number from urine followed by pus, wound, aspirates, blood, ear, stool, and the least from sputum. Among 180 isolates, 105 isolates were obtained from in-patients samples and 75 isolates were isolated from out-patients samples.

Table 3 shows the antimicrobial susceptibility of all *E. coli* isolated from urine, pus, and blood. In our study, it is observed that *E. coli* is 100% susceptible to imipenem. Susceptibility to third generation cephalosporin is between 30 and 35%, which is quite low. Susceptibility to piperacillin/tazobactam, amoxicillin/clavulanic acid, cefoperazone/sulbactam, ceftazidime/clavulanate, amikacin, norfloxacin (only in urine), and ciprofloxacin was good and between 50 and 90%. Third generation cephalosporin resistant *E. coli* from various samples shows better susceptibility to antibiotics with ESBL inhibitor combination.

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

Of the total *E. coli* isolates, 100 (55.55%) isolates were ESBL producers and 80 (44.45%) isolates were non-ESBL producers. Among ESBL producers, the maximum number was isolated from blood (66.67%), followed by aspirate (65%), stool (57.14%), wound (55%), and urine (54.67%) (Table 4).

Of the 105 organisms isolated from in-patients, 64 (60.95%) were ESBL producers while 36 (48%) out of 75 from out-patients were ESBL producers. ESBL producers were more common among in-patients than out-patients. ESBL and non-ESBL producers compared among in- and out-patients give significant result ($P < 0.001$) (Table 5).

Antimicrobial sensitivity pattern of ESBL producing *E. coli* from urine and blood showed that it was 100% susceptible to imipenem, but susceptibility to third generation cephalosporin and non-β-lactam antibiotic was further decreased as compared to non-ESBL producing *E. coli* (Table 6). Susceptibility to ESBL inhibitor combination drugs was almost the same as compared to non-ESBL producing *E. coli*.

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 ceftazidime/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 (cefoxitin) (31.11%), monobactam (aztreonam) (31.11%), piperacillin (33.33%), cefoperazone (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 NO. (%)	n = 25 SENSITIVE NO. (%)	n = 15 SENSITIVE NO. (%)	n = 180 SENSITIVE 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 1 (84%)	13 (86.66%)	157 (87.22%)
Amoxicillin/Clavulanic acid	60 (80%)	18 (72%)	11 (73.33%)	136 (75.55%)
Cefoperazone/Sulbactam	62 (82.66%)	20 (80%)	13 (86.66%)	138 (76.67%)
Ceftazidime/Clavulanate	57 (76%)	18 (72%)	11 (73.33%)	119 (66.11%)
Cephems (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%)
Ceftriaxone	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/sulbactam, 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 *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.[14]

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

ESBL producing *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 *E. coli* isolates were wide spread among both in-patients and

Table 6. Antimicrobial susceptibility pattern of ESBL producing *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%)
Amoxycillin/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%)
Cefoxitin	5 (12.19%)	3 (30%)
Ceftazidime	9 (21.95%)	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%)
Flouroquinolones		
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 aminoglycosides 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.

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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