

Extended Spectrum Beta-Lactamase Producing Organisms and their Antibiotic Resistance among Study Population: A Clinical Study

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ABSTRACT

Introduction: Extended spectrum beta-lactamase (ESBL) producing organisms hydrolyze the oxyimino beta-lactams and monobactams. The emergence of these organisms poses major difficulty in treating infections. The present study was conducted to identify antibiotic resistance profile of Extended spectrum beta-lactamase (ESBL) in the urinary tract infections (UTI).

Material and Methods: The present study was conducted in the Department of Microbiology in year 2015. It consisted of 520 patients. Identification of bacterial growth was confirmed by standard microbiological and biochemical techniques. Patients were also subjected for antibiotic sensitivity testing (AST). It was done with Kirby-Bauer disc-diffusion method on Mueller-Hinton agar. We determine the susceptibility to an antibiotic for particular organism. The control strains used were *Escherichia coli* ATCC and *Pseudomonas aeruginosa* ATCC. Extended spectrum beta-lactamase confirmatory tests were also done. Increase of ≥ 5 mm in the zone diameter of ceftazidime/clavulanic acid disc with respect to that of ceftazidime disc alone and or ≥ 5 mm increase in the zone diameter of cefotaxime/clavulanic acid disc with respect to that of cefotaxime disc alone were indicative ESBL producer. *Escherichia coli* 25922 and a known in-house ESBL producer were used as negative and positive controls respectively. Once the ESBL producers were isolated, their antibiotic resistance profile were prepared and evaluated.

Results: Out of 520 patient urine samples, 208 (40%) was found positive which included 240 were males and 280 were females. Out of which 120 males (50%) and 88 females (31%) were found positive. Of 220 pathogens identified in 208 subjects, gram positive was seen in 22 (10%), gram negative in 165 (75%) and candida in 33 (15%). The difference was significant ($P < 0.05$). Out of 165 gram negative, 66 (40%) were ESBL positive, males (75) and females (90). Out of which 21 males and 45 females found to be ESBL positive. Gram positive were seen in 10 males and 11 females. Candida was seen in 13 males and 20 females. We also recorded different gram negative species with ESBL positive cases. Out of 66, *E. coli* (32), *Klebsiella pneumoniae* (24), *Pseudomonas aeruginosa* (4), *Actinobacter baumannii* (3), *Proteus mirabilis* (2) and *Enterobacter cloacae* (1) were seen. The difference was significant ($P < 0.05$). We recorded antibiotic resistance profile of various ESBL and non ESBL. This includes amikacin, gentamycin, ampicillin, tetracycline, colistin, norfloxacin, levofloxacin, cotrimoxazole, ceftazidime and cefepime.

Conclusion: The emergence of various ESBL led to failure treatment of infections. The antibiotic sensitivity should be routinely done procedure in order to prevent complications and failure of treatment.

Keywords: Ampicillin, Cefepime, Extended spectrum beta-lactamase, Norfloxacin

acts against both Gram-positive and Gram-negative bacteria, whereas narrow-spectrum antibiotic, is effective against specific families of bacteria. An example of a commonly used broad-spectrum antibiotic is ampicillin.¹

The term 'broad-spectrum antibiotic' was used in the mid-1950s, when the bacterial spectrum of chloramphenicol and the first tetracyclines could be strikingly opposed to the narrow spectrum of activities of penicillin G, and streptomycin. In the 1960s, amino penicillins, then ureidopenicillins, became the broad spectrum penicillins in comparison with penicillin G.²

Extended spectrum beta-lactamase (ESBL) producing organisms hydrolyze the oxyimino beta-lactams and monobactams. The emergence of these organisms poses major difficulty in treating infections. They are increasing rapidly and their detection and treatment is difficult thus causing increase mortality of patients.³ The incidence of ESBL producing organisms is rapidly changing with time. Their occurrence also varies from place to place. The endemicity of ESBL producers have resulted in outbreaks of infection in various hospitals worldwide. Antibiotics used against various ESBL producers leads to failures and increased patient mortality.⁴

The ESBL producers also have resistance to other antibiotic groups. Thus there remain few antibiotics effective against these agents. A delay in appropriate therapy can cause severe complications. Detection of ESBL producers from sample such as urine may be of utmost importance because this represents an epidemiologic marker of colonization and therefore there is potential for transfer of such organisms to other patients. The rapid increase of resistance to broad spectrum beta lactams among uropathogens has recently become a major problem globally. It leads to antibiotic ineffectiveness, increased severity of illness and cost of treatment.⁵ The serious increase in the prevalence of ESBL's worldwide creates a need for effective and easy to perform screening methods for detection. The present study was conducted to identify antibiotic resistance profile of Extended spectrum beta-lactamase (ESBL) in the urinary tract infections (UTI).

MATERIAL AND METHODS

The present study was conducted in the Department of Microbiology in year 2015 in TSM medical college and hospital,

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How to cite this article: Rajesh Kumar Yadav. Extended spectrum beta-lactamase producing organisms and their antibiotic resistance among study population: a clinical study. International Journal of Contemporary Medical Research 2016;3(12):3502-3504.

Lucknow. It consisted of 520 subjects and their urine samples were collected in sterile containers. Calibrated loop method on a UTI chromogenic media was used for semi-quantitative culture of urine. The culture plates were incubated at 37°C for 18-24 h under aerobic conditions. Identification of bacterial growth was confirmed by standard microbiological and biochemical techniques. Patients were also subjected for antibiotic sensitivity testing (AST). It was done with Kirby-Bauer disc-diffusion method on Mueller-Hinton agar. Mueller-Hinton agar was uniformly and aseptically inoculated with the test organism and then filter paper discs, which were impregnated with a specific concentration of a particular antibiotic, were placed on the medium. "Zone of inhibition" refers to where there was no growth around the disc containing the antibiotic. It was observed and measured to determine the susceptibility to an antibiotic for that particular organism. The antibiotics like Amikacin (30 mcg), gentamycin (10 mcg), ampicillin (10 mcg), levofloxacin (5 mcg), norfloxacin (10mcg), tetracycline (30 mcg), cefazolin (30 mcg), cefepime (30 mcg) and cotrimoxazole (1.25/23.75 mcg) were tested. The control strains used were *Escherichia coli* ATCC and *Pseudomonas aeruginosa* ATCC. Extended spectrum beta-lactamase confirmatory tests were also done. While performing antibody sensitivity test (AST), ceftazidime plus clavulanic acid (30/10 mcg) and cefotaxime plus clavulanic acid (30/10 mcg) discs were also included along with ceftazidime (30 mcg) and cefotaxime (30 mcg) discs on Muller-Hinton agar. Increase of ≥ 5 mm in the zone diameter of ceftazidime/clavulanic acid disc with respect to that of ceftazidime disc alone and or ≥ 5 mm increase in the zone diameter of cefotaxime/clavulanic acid disc with respect to that of cefotaxime disc alone were indicative ESBL producer. *Escherichia coli* 25922 and a known in-house ESBL producer were used as negative and positive controls respectively. Once the ESBL producers were isolated, their antibiotic resistance profile were prepared and evaluated.

STATISTICAL ANALYSIS

SPSS version 21 was used for the statistical analysis. Descriptive statistics like mean and percentages were used to interpret the results.

RESULTS

Table 1 shows that out of 520 patient urine samples, 208 (40%) was found positive. Table 2 shows that out of out of 520 tested patients, 240 were males and 280 were females. Out of which 120 males (50%) and 88 females (31%) were found positive. Of 220 pathogens identified in 208 subjects, gram positive was seen in 22 (10%), gram negative in 165 (75%) and candida in 33 (15%). The difference was significant ($P < 0.05$). Figure-2 shows that out of 165 gram negative, 66 (40%) were ESBL positive. Figure-3 shows that out of 165 gram negative, males were 75 and females were 90. Out of which 21 males and 45 females found to be ESBL positive. Figure-4 shows that gram positive were seen in 10 males and 11 females. Candida was seen in 13 males and 20 females. Figure-5 shows different gram negative species with ESBL positive cases. Out of 66, *E coli* (32), *Klebsiella Pneumonia* (24), *Pseudomonas Aerogenosa* (4), *Actinobacter Baumanni* (3), *Proteus Mirabilis* (2) and *Enterobacter Cloacae* (1) were seen. The difference was significant ($P < 0.05$). Figure-6 shows antibiotic resistance profile of various ESBL and non ESBL. This includes amikacin, gentamycin, ampicillin,

tetracycline, colistine, norfloxacin, levofloxacin, cotrimoxazole, ceftazidime and cefepime.

DISCUSSION

The present study was conducted to identify antibiotic resistance

Total	Prevalence	Percentage
520	208	40%

Table-1: Distribution of patients

Total - 520		
Gender	Male	Female
Total	240	280
Positive culture	120	88
Percentage	50%	31%

Table-2: Gender distribution of patients

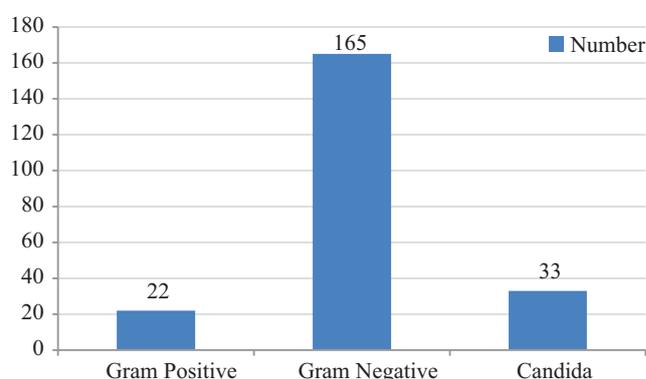


Figure-1: Distribution of gram positive and gram negative pathogens

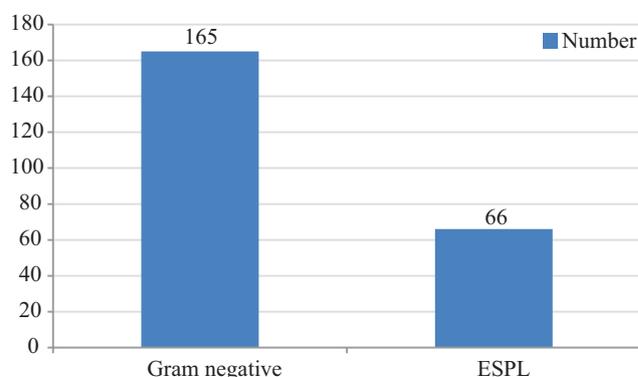


Figure-2: Gram negative species

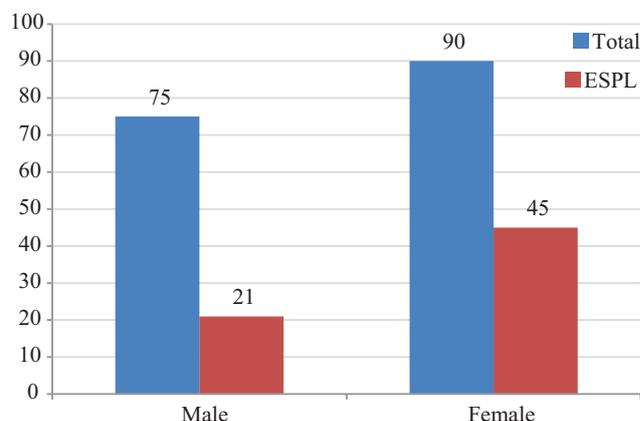


Figure-3: Prevalence of ESBL in gram negative species according to gender

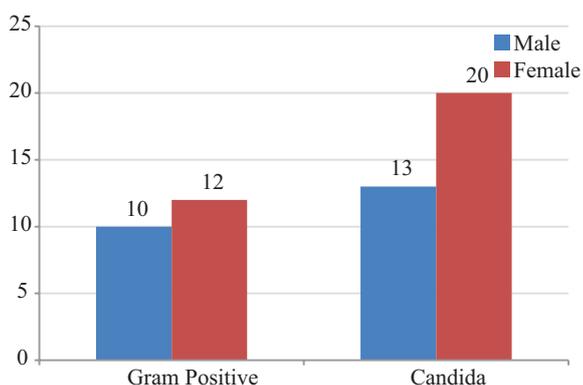


Figure-4: Gram positive and Candida species according to gender

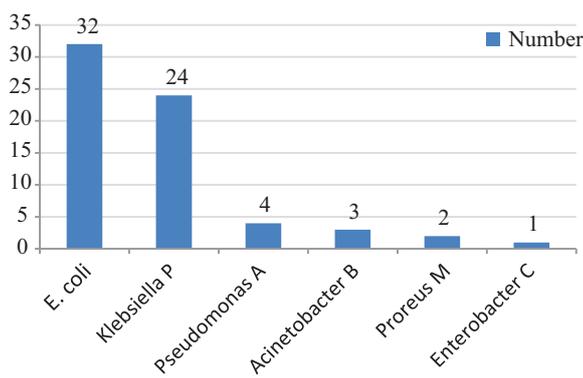


Figure-5: Different gram negative species

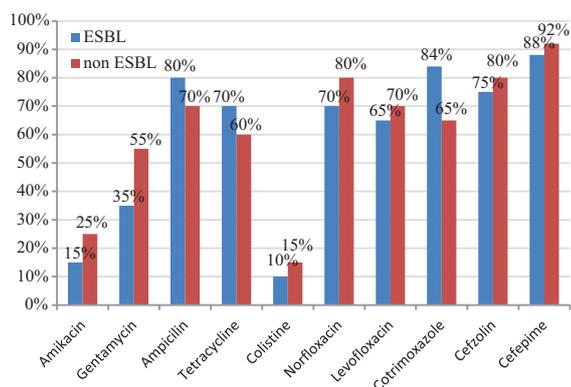


Figure-6: Antibiotic resistance

profile of Extended spectrum beta-lactamase (ESBL) in the urinary tract infections (UTI) and their prevalence in study population. It consisted of 520 subjects. Patients were also subjected for antibiotic sensitivity testing (AST). The following antibiotics were tested: Amikacin (30 mcg), gentamycin (10 mcg), ampicillin (10 mcg), levofloxacin (5 mcg), norfloxacin (10mcg), tetracycline (30 mcg), cefazolin (30 mcg), cefepime (30 mcg) and cotrimoxazole (1.25/23.75 mcg). Extended spectrum beta-lactamase confirmatory tests were also done. Out of 520 patient urine samples, 208 (40%) was found positive. Our results are in agreement with the results of Coolee et al.⁶ The present study included 240 were males and 280 were females. Out of which 120 males (50%) and 88 females (31%) were found positive. Of 220 pathogens identified in 208 subjects, gram positive was seen in 22 (10%), gram negative in 165 (75%) and candida in 33 (15%). Willinger⁷ in his study found 34% gram positive and 45% gram negative isolates. We found that out of 165 gram negative, 66 (40%) were ESBL

positive which comprised of 75 males and 90 females. 21 males and 45 females found to be ESBL positive. Our results are in agreement with Sood S et al.⁸ Gram positive were seen in 10 males and 11 females. Candida was seen in 13 males and 20 females. We found that out of 66, E coli (32), Klebsiella Pneumonia (24), Pseudomonas Aerogenosa (4), Actinobacter Baumanni (3), Proteus Mirabilis (2) and Enterobacter Cloacae (1) were seen.

We also recorded antibiotic resistance profile of various ESBL and non ESBL. This includes amikacin, gentamycin, ampicillin, tetracycline, colistin, norfloxacin, levofloxacin, cotrimoxazole, ceftazidime and cefepime. Bauer AW⁹ and others¹⁰ also tested antibiotic resistance of antibiotics in his study and found same results.

CONCLUSION

The emergence of various ESBL led to failure treatment of infections. The antibiotic sensitivity should be routinely done procedure in order to prevent complications and failure of treatment.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 19-11-2016; **Published online:** 31-12-2016