

## Evaluation of extended spectrum beta lactamase in urinary isolates

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Received December 9, 2003

**Background & objectives:** Urinary tract infection (UTI) remain the common infections diagnosed in outpatients as well as in hospitalized patients. Current knowledge on antimicrobial susceptibility pattern of uropathogens is mandatory for appropriate therapy. Extended spectrum beta lactamases (ESBL) hydrolyse expanded spectrum cephalosporins like ceftazidime, cephotaxime which are used in the treatment of UTI. ESBL producing bacteria may not be detectable by routine disk diffusion susceptibility test, leading to inappropriate use of antibiotics and treatment failure. Not much information on ESBL producing organisms causing UTI is available from India. An effort was therefore made to study the ESBL producing uropathogens and also the susceptibility patterns of ESBL and nonESBL producers.

**Methods:** Urinary isolates from symptomatic UTI cases attending or admitted to the Indira Gandhi Medical College and Hospital, Nagpur were identified by conventional methods. Antimicrobial susceptibility testing was done by Kirby Bauer's disc diffusion method. Isolates resistant to cephotaxime were tested for ESBL production by double disc synergy test method.

**Results:** Of the 217 isolates, 87 were cephotaxime resistant Gram-negative bacilli. Of these, 42 (48.3%) were found to be ESBL producers. *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter* were ESBL producing species. Multidrug resistance was found to be significantly ( $P < 0.05$ ) more in ESBL producing isolates (90.5%) than non ESBL producers (68.9%).

**Interpretation & conclusion:** In the present study a large number of uropathogens were found to be ESBL producers. Most of the ESBL producing isolates were multidrug resistant. Monitoring of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with UTI.

**Key words** Extended spectrum beta lactamases - uropathogen

Despite the widespread availability of antibiotics, urinary tract infection (UTI) remains the most common bacterial infection in the human population. Antibiotics are usually given empirically before the laboratory results of urine culture are available. To

ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory<sup>1</sup>. Extended spectrum beta lactamases (ESBL) hydrolyse oxyimino beta lactams like ceftazidime, cephotaxime, ceftriaxone and

monobactam but have no effect on cephamycins carbapener and related compounds<sup>2</sup>. They arise by mutations in genes for common plasmid-mediated beta lactamases that alter the configuration of the enzyme near its active site to increase the affinity and hydrolytic ability of the beta lactamases for oxyimino compounds while simultaneously weakening the overall enzyme efficiency. Some ESBLs confer high level resistance to all oxyimino beta lactams, but for other ESBLs resistance is only slightly increased or increased selectively for particular beta lactams. This creates a problem for the clinical laboratory, since organisms producing less active ESBLs can fail to reach current National Committee for Clinical Laboratory Standard's (NCCLS) break points for resistance but can cause significant disease<sup>3-5</sup>. Recent studies on ESBL production in members of Enterobacteriaceae isolated from clinical specimens showed 9-50 per cent ESBL producers<sup>6-8</sup>. A study from north India on ESBL production in uropathogens showed 26.6 per cent ESBL producers which belonged to *Klebsiella*, *Escherichia coli*, *Enterobacter*, *Proteus* and *Citrobacter* species<sup>9</sup>.

With reports on high prevalence of ESBL production in members of Enterobacteriaceae and paucity of information specially on uropathogens from our country, the present study was undertaken to evaluate the ESBL producing uropathogens in patients attending a tertiary care hospital at Nagpur as also the differences between the antimicrobial susceptibility patterns of ESBL and non ESBL producers.

### Material & Methods

During mid January to September 2003, a total of 1799 urine samples were received in the Department of Microbiology, Indira Gandhi Medical College and Mayo Hospital, Nagpur. From these urine samples of symptomatic UTI patients 217 urinary isolates were identified by conventional techniques<sup>10-12</sup>.

Antibiogram of the isolates was done by Kirby Bauer's Method<sup>13</sup> using antibiotic disks from Hi-media, Mumbai. Antibiotics used for Gram-negative bacilli were ampicillin (10 µg), co-trimoxazole (25 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), cephotaxime (30 µg) and

amikacin (10 µg). For *Staphylococcus* penicillin (10unit), oxacillin (1 µg) and vancomycin (30 µg) were also used. The results were interpreted as per National Committee for Clinical Laboratory Standard (NCCLS) recommendations<sup>14</sup>.

Eighty seven isolates of Gram-negative bacteria with resistance or with decreased susceptibility (intermediate by NCCLS criteria) to third generation cephalosporins were tested for ESBL production by double disk synergy test (DDST)<sup>15</sup>. A lawn culture of test strain on Muller Hinton agar (Hi-media, Mumbai) was exposed to disc of cephotaxime (30 µg) and a disc of amoxiclav (augmentin) (20 µg amoxicillin/10 µg clavulanic acid) arranged in pairs. The discs were arranged so that the distance between them was approximately twice the radius of the inhibition zone produced by cephotaxime tested on its own. The test isolate was considered to produce ESBL, if the zone size around the antibiotic disc increased towards the augmentin disc<sup>15</sup>. Test of proportion was used for data analysis.

### Results & Discussion

Various organisms have been reported to be isolated from patients with UTI. *E. coli*<sup>9,16,17</sup> and *Klebsiella*<sup>18</sup> have been reported as the most common organisms causing UTI.

In the present study, of the 217 uropathogens, 108 (49.8%) isolates of *E. coli* were found to be the most common organisms followed by *Klebsiella pneumoniae* (82, 37.8%) *Pseudomonas aeruginosa* (14, 6.5%), *Staphylococcus saprophyticus* (4, 1.84%), *Acinetobacter* species (3, 1.4%) and *Proteus mirabilis* and *Staphylococcus aureus* (2, 0.9%) each. One strain each of *Candida albicans* and *Enterobacter* species was also isolated.

Of 216 bacterial uropathogens studied, antibiogram revealed that 177 (82%) and 172 (79.6%) isolates to be resistant to co-trimoxazole and ampicillin, respectively indicating maximum resistance to these drugs. Nitrofurantoin, cephotaxime and norfloxacin constitute the reasonable option for treatment of UTI as 135 (62.5%), 127 (58.7%) and 97 (44.9%) isolates were sensitive to these antibiotics

respectively. Amikacin was found to be drug of choice for treatment of UTI caused by *Pseudomonas*, as 12 (85.7%) isolates were sensitive to amikacin. A total of 144 (73.5%) Gram-negative bacilli were resistant to nalidixic acid.

Of the 216 bacterial uropathogens isolated, 89 (41%) showed resistance to cephalexin (including two isolates of *S. saprophyticus*). Of these, 87 cephalexin-resistant Gram-negative bacilli were tested for ESBL production and 42 (48.3%) were found to be ESBL producers.

High prevalence rate of ESBL producing strains have been reported in *Klebsiella* species<sup>7,16,17,19</sup>. In the present study, 21 (25.6%) *Klebsiella* isolates were ESBL producers. ESBL production in *E. coli* has been reported to vary from 21 to 34 per cent<sup>7,16,17,20</sup>. Twenty (18.5%) *E. coli* isolates ESBL producers in the present study. One isolate of the *Acinetobacter* was found to produce ESBL.

The production of beta-lactamase may be of chromosomal or plasmid origin<sup>21</sup>. Plasmid mediated production is often acquired by transfer of genetic information from one organism to another. Such transferable plasmid also codes for resistant determinants to other antimicrobial agents. Hence multidrug resistance is expected to be more common in ESBL producing organisms. In the present study, 38 (90.5%) ESBL producing isolates were found to be resistant to three or more drugs whereas multidrug resistance in non ESBL producers was seen in only 31 (68.9%) isolates. The difference was statistically significant ( $P < 0.05$ ).

Our study showed that ESBL production was high among uropathogens. The situation is worsened due to increased multidrug resistance seen in ESBL producers than in non ESBL producers. Hence, routine ESBL testing for uropathogens along with conventional antibiogram would be useful for all cases of UTI.

## References

1. Grubenberg GN. Antibiotic sensitivities of urinary pathogens: 1971-1982. *Antimicrob Chemother* 1984; 14 : 17-23.
2. Philippon A, Labia R, Jacoby G. Extended spectrum beta lactamases. *Antimicrob Agents Chemother* 1989; 33 : 1131-6.
3. Katsanis GP, Spargo J, Ferraco MJ, Sutton L, Jacoby GA. Detection of *Klebsiella pneumoniae* and *Escherichia coli* strains producing extended spectrum beta lactamases. *J Clin Microbiol* 1994; 32 : 691-6.
4. Meyer KS, Urban C, Eagan JA, Berger BJ, Rahal JJ. Nosocomial outbreak of *Klebsiella* infection to late generation cephalosporins. *Annu Intern Med* 1993; 119 : 353-8.
5. Phillipon A, Fournier G, Paul G, Vedel G, Nevot P. Detection of distribution des beta lactamases a spectre elargi chez les enterobacteries. *Med Mal Infect* 1988; 12 : 869-79.
6. Quinteros M, Radice M, Gardella N, Rodriguez MM, Costa N, Korbenfeld D, *et al*. Extended spectrum beta lactamases in Enterobacteriaceae in Buenos Aires, Argentina, Public Hospitals. *Antimicrob Agents Chemother* 2003; 47 : 2864-7.
7. Akata F, Tatman-Otkum M, Ozkan E, Tansel O, Otkum M, Tugrul M. Prevalence of extended spectrum beta lactamases produced by nosocomial isolates of enterobacteriaceae in Trakta University Hospital, Turkey. *New Microbiol* 2003; 26 : 257-62.
8. Hansotia JB, Agrawal V, Pathak AA, Saoji AM. Extended spectrum beta lactamases mediated resistance to third generation cephalosporins in *Klebsiella pneumoniae* in Nagpur, Central India. *Indian J Med Res* 1997; 105 : 156-61.
9. Khurana S, Taneja N, Sharma M. Extended spectrum beta lactamases mediated resistance in urinary tract isolates of family enterobacteriaceae. *Indian J Med Res* 2002; 116 : 145-9.
10. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. The Enterobacteriaceae. In: *Color atlas & text book of diagnostic microbiology*, 5th ed. Philadelphia: JB Lippincott Co.; 1997 p.171-230.
11. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. The nonfermentative Gram negative bacilli. In: *Color atlas & text book of diagnostic microbiology*, 5th ed. Philadelphia: JB Lippincott Co.; 1997 p. 253-303.
12. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. The Gram positive cocci: Part I: Staphylococci and related organisms. In: *Color atlas & text book of diagnostic microbiology*, 5th ed. Philadelphia: JB Lippincott Co.; 1997 p. 550-66.

13. Bauer AW, Kirby WMM, Sherris JC, Tuck M. Antibiotics susceptibility testing by a standardized single disc method. *Am J Clin Pathol* 1966; 45 : 493-6.
14. National Committee for Clinical Laboratory Standards. Performance standard for antimicrobial disc susceptibility test, 5th ed. Villanova PA: NCC1993; Document M2-A5.
15. Miles RS, Amyes SGB. Laboratory control of antimicrobial therapy. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie and McCartney Practical Medical Microbiology*, 14th ed. New York: Churchill Livingstone; 1996 p. 167.
16. Gupta V, Yadav A, Joshi RM. Antibiotic resistance pattern in uropathogen. *Indian J Med Microbiol* 2002; 20 : 96-8.
17. Gales AC, Sader HS, Jones RN, SENTRY participants group (Latin America). Urinary tract infection's trends in the American hospitals: reports from the SENTRY Antimicrobial Surveillance programme (1997-2000). *Diagn Microbiol Infect Dis* 2002; 44 : 289-99.
18. Bajaj JK, Karyokart RP, Kulkarni JD, Deshmukh AB. Changing aetiology of urinary tract infections and emergence of drug resistance as major problem. *J Commun Dis* 1999; 31 : 181-4.
19. Mathur P, Tatman A, Das B, Dhawan B. Prevalence of extended spectrum beta lactamase producing gram negative bacteria in a tertiary care hospital. *Indian J Med Res* 2002; 115 : 153-7.
20. Iqbal M, Patel IK, Shal SH, Ain Q, Barrey N, Kiani Q, et al. Susceptibility patterns of *Escherichia coli* prevalence of multidrug resistant isolates and extended spectrum beta lactamase phenotype. *J Pak Med Assoc* 2002; 52 : 407-11.
21. Yu Y, Zhou W, Chen Y, Ma Y. Epidemiological and antibiotic resistance study on extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in Zhejiang province. *Clin Med J* 2002; 115 : 1479-82.

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Extended-spectrum beta-lactamases (ESBLs) are beta-lactamases that hydrolyze penicillins, cephalosporins, and aztreonam; do not hydrolyze cephamycins; and are inhibited by beta-lactamase inhibitors. Beyond that, ESBL-producing Enterobacteriaceae (ESBL-E) are frequently resistant to other antibiotics. ESBL-E have dramatically spread worldwide during the last two decades. The fact that carbapenems have been considered the preferred option for invasive infections due to ESBL-E has fueled the use of these drugs, both empirically and whenever an ESBL-E is isolated, which is contributing to the spread. ARTICLE Evaluation of extended-spectrum beta-lactamase production in *Escherichia coli* clinical isolates from three hospitals in Luzon, Philippines Merlyn C. Cruz\*<sup>1,2</sup>, Catherine S. Bacani<sup>1</sup>, Alma B. Mendoza<sup>1</sup>, and Cynthia T. Hedreya<sup>2</sup> <sup>1</sup> Angeles University Foundation, Angeles City National Institute of Molecular Biology and Biotechnology, University of the Philippines, Diliman Quezon City <sup>2</sup> The increased spread of pathogens producing extended-spectrum beta-lactamase (ESBL) enzyme among members of family Enterobacteriaceae has been a serious problem worldwide. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 2001; 14 (4):933-951.