Extended-spectrum beta-lactamases in urinary isolates of Escherichia coli, Klebsiella pneumoniae and other gram-negative bacteria in a hospital in Eastern Province, Saudi Arabia

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ABSTRACT

Objective: To evaluate the prevalence and antimicrobial susceptibility of extended spectrum beta-lactamase (ESBL) producing gram-negative organisms isolated from patients with urinary tract infection (UTI).

Methods: We carried out this study at Almana General Hospital, Eastern Province, Kingdom of Saudi Arabia, during the period August 2003 to October 2004. We studied urinary isolates of *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterobacter spp* and *Pseudomonas aeruginosa* (*P. aeruginosa*) for ESBL production and antimicrobial susceptibility.

Results: We studied a total of 2302 urinary gram-negative isolates for the presence of ceftazidime resistance and ESBL. The isolates included *E. coli* (1238), *K. pneumoniae* (522), *Enterobacter spp* (138) and *P. aeruginosa* (404). Of the 2302 isolates, 232 (10%) were ceftazidime resistant and 204 (8.9%) were ESBL producers. We detected ESBL in 119 (9.6%) *E. coli*, 59 (11.3%) *K. pneumoniae*, 14 (10.14%) *Enterobacter species* and 12 (2.97%) *P. aeruginosa* isolates. The ESBL-producing strains were most commonly isolated from patients with indwelling Foley's catheter [131 (64.2%)] and those in the long-term care ward [90 (44.2%)]. Only 26 (12.7%) ESBL-producing isolates were from outpatients. More than 89% of the ESBL producers were susceptible to imipenem and meropenem. Amikacin and piperacillin/tazobactam were active against 68% and 45% of the isolates. Susceptibility to gentamicin and ciprofloxacin was 22.5% and 14%. The least active antibiotic was cefepime (11.8%).

Conclusion: This study shows the presence of ESBL producers in uropathogens from both inpatients and outpatients and demonstrates their high resistance to various classes of antimicrobial agents.

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Infections by extended-spectrum beta-lactamase (ESBL)-producing organisms are causing a growing worldwide problem and significant diagnostic and therapeutic challenges.[1,2] Most ESBLs are mutant forms of TEM and sulphydryl variable (SHV) enzymes coded by genes located on plasmids that can be easily spread from one organism to another.[3] These enzymes are capable of inactivating a variety of ß-lactam drugs, including third-generation cephalosporins, extended-spectrum penicillins and monobactams.[4] The ESBL-producing organisms are often multidrug resistant, as the plasmids producing ESBLs can carry resistance to other antibiotics.[5,6] Due to cross-resistance to other antibiotics, the therapeutic choices in infections caused by ESBL-producing organisms are limited.[7]

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The increasing antimicrobial resistance among ESBL-producing bacteria causing urinary tract infection (UTI) makes therapy of UTI difficult and leads to more use of expensive broad-spectrum drugs such as carbapenems, which are known to be the most active antibiotics against these organisms.[7] Additionally, routine in vitro susceptibility testing may not detect these resistant isolates, which can result in adverse therapeutic outcomes.[8] Antimicrobial susceptibility of bacterial isolates from UTIs differs from country to country and from region to region. Antimicrobial surveillance studies can be used to assess the changes in patterns of susceptibility of bacterial pathogens to antimicrobial agents. As there are not enough local data on the prevalence of ESBL producers in UTI and as these data affect the choices for empirical therapy of UTI, we conducted this study to determine the prevalence of ESBL producers in urinary isolates of *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae (K. pneumoniae*), *Enterobacter species* and *Pseudomonas aeruginosa* (*P. aeruginosa*), and analyzed the patterns of their susceptibility to various antimicrobial agents.

Methods. Bacterial isolates studied were *E. coli* (1238), *K. pneumoniae* (522), *Enterobacter spp* (138) and *P. aeruginosa* (404). Isolates were identified by standard techniques and the API 20E (BioMerieux, France).[9] A total of 232 ceftazidime resistant urinary isolates from patients at Almana General Hospital, Khobar and Dammam, Kingdom of Saudi Arabia, were tested for ESBL production. The study period was from August 2003 to October 2004. Clinical and demographic data of study patients were noted. Testing for ESBL production was carried out by double-disc diffusion tests using separate commercial discs containing cefotaxime (30 mcg) and ceftazidime (30 mcg) with and without clavulanic acid (10 mcg) (Mast Diagnostics,

Table 1 - The species distribution for extended spectrum ß-lactamase (ESBL) producing organisms.

Organisms	n of isolates	ESBL strains n	ESBL strains (%)
E. coli	1238	119	(9.6)
K. pneumoniae	522	59	(11.3)
Enterobacter spp	138	14	(10.1)
P. aeruginosa	404	12	(2.9)
Total	**2302**	**204**	**(8.9)**

E - Escherichia, K -Klebsiella, spp - Species, P - Pseudomonas

Table 2 - Number and percentage of extended spectrum ß-lactamase (ESBL) producing organisms.

Diagnosis	ESBL strains n	(%)
UTI following indwelling Foley' catheter	**131**	**(64.2)**
Long term care	90	(44.2)
Dialysis unit	9	(4.4)
Medical unit	16	(7.8)
Renal transplant patients	16	(7.8)
UTI following pre and post operative catheter insertion	**24**	**(11.8)**
Cesarean section	7	(3.4)
Pelvic floor repair	7	(3.4)
Cholecystectomy	3	(1.5)
Appendicetomy	3	(1.5)
Orthopedic surgery	2	(1)
TURP	2	(1)
UTI following ureteric instrumentation	**23**	**(11.3)**
Ureteroscopy	12	(5.9)
Ureteric catheterization	1	(0.5)
Ureteric stenting	10	(4.9)
UTI in outpatient unit	**26**	(12.7)
Burning micturition / dysuria	17	(8.3)
Lower abdominal pain	9	(4.4)
Total	**204**	**(100)**

UTI - urinary tract infection,
TURP - transurethral resection of prostate

Table 3 - Empirical antimicrobial therapy of patient positive for extended spectrum ß-lactamase (ESBL) producing organisms.

Therapy	n	(%)
Monotherapy		
Ciprofloxacin	58	(28.4)
Ceftazidime	25	(12.3)
Meropenem	16	(7.8)
Imipenem	6	(2.9)
Piperacilln/Tazobactam	13	(6.4)
Gentamicin	9	(4.4)
Cefepime	6	(2.9)
Combined therapy		
Ciprofloxacin + gentamicin	15	(7.4)
Ceftazidime + gentamicin	12	(5.9)
Imipenem + gentamicin	7	(3.4)
Ceftazidime + ciprofloxacin	6	(2.9)
Cefepime + ciprofloxacin	5	(2.5)
Cefepime + gentamicin	4	(2)
No antibiotic therapy	22	(10.8)
Total	**204**	**(100)**

Table 4 - Number and percentage of antibiotics active against extended spectrum ß- lactamase (ESBL) - producing organisms.

Organisms	isolates n	MEP n	MEP (%)	IMP n	IMP (%)	AK n	AK (%)	PIP/TAZ n	PIP/TAZ (%)	GN n	GN (%)	CIP n	CIP (%)	CEP n	CEP (%)
E. coli	119	110	(92)	110	(92)	81	(68)	46	(38.7)	28	(23.5)	14	(11.8)	12	(10)
K. pneumoniae	59	54	(91.5)	54	(91.5)	40	(67.7)	30	(50.8)	11	(18.6)	10	(16.9)	8	(13.5)
Enterobacter spp	14	13	(92.8)	13	(92.8)	9	(64)	9	(64)	1	(7)	3	(21)	3	(21)
P. aeruginosa	12	8	(66.6)	6	(50)	9	(75)	7	(58)	6	(50)	2	(16)	1	(8)
Total	**204**	**185**	**(90)**	**183**	**(89.7)**	**139**	**(68)**	**92**	**(45)**	**46**	**(22.5)**	**29**	**(14)**	**24**	**(11.8)**

MEP - meropenem, IMP - imipenem, AK - amikacin, PIP/TAZ - piperacillin-tazobactam, GN - gentamicin, CIP - ciprofloxacin, CEP - cefepime, *E - Escherichia, K - Klebsiella, spp - species, P - Pseudomonas.*

Merseyside, United Kingdom).[10] A 5 mm or more increase in the zone size for cefotaxime and ceftazidime with and without clavulanic acid was considered to indicate ESBL production. Strains of *E. coli* (ATCC 25922) as a negative control and *K. pneumoniae* (ATCC 700603) as a positive control were included. Minimum inhibitory concentration (MIC) for ESBL-producing bacteria against the above antibiotics was performed with the agar dilution methods recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[11]

Results. Of the 2302 urinary isolates studied, 232 (10%) were ceftazidime resistant and 204 (8.9%) were ESBL producers. The species distribution for ESBL-producing bacteria is shown in **Table 1**. The patients positive for ESBL-producing bacteria were 26 (12.7%) outpatients and 178 (87%) inpatients, including 90 (44%) from long-term care ward. Of the 204 patients, 131 (64.2%) had indwelling Foley's catheter. Details of clinical conditions associated with positive ESBL isolates are listed in **Table 2**. One hundred and eighty-two (89%) patients positive for ESBL producers were receiving one or more antibiotics empirically. Thirty-one (21%) patients were receiving ceftazidime either alone or in combination with another antibiotic. Details of empirical antimicrobial therapy are listed in **Table 3.** Carbapenems (imipenem and meropenem) demonstrated susceptibility greater than 89% against all the ESBL-producing isolates. The next most active antibiotics were amikacin (68%) and piperacillin/tazobactam (45%). The susceptibility to gentamicin was 22.5% and to ciprofloxacin was 14%. Cefepime was active only against 11.8% of the isolates. The antimicrobial susceptibility results of ESBL producers are shown in **Table 4.**

Discussion. The prevalence of ESBL-producing clinical isolates varies from one country to another and is rapidly changing over time.[1] Our study showed the occurrence of ESBL-producing strains in urinary isolates of *E. coli* was 9.6% and in *K. pneumoniae* was 11.3%. This is higher than the reported figures of *E. coli* and *K. pneumoniae* in Canada (2.7-6.2%) and United State of America[12] (2.2-6.6%), and is lower than those reported in India[13] (41-40%). Several case control studies have reported the risk factors associated with acquisition of ESBL-producing *E. coli* and *K. pneumoniae*.[14,15] The risk factors listed were ceftazidime or aztreonam exposure, the presence of instrumentation and residence in a nursing home. In another study,[16] the following risk factors were noted: prior antibiotics, presence of a central venous catheter or urinary catheter, nosocomial infection and duration of hospitalization before infection. In our study, 89% of the patients infected with ESBL-producing bacteria had received antimicrobial therapy, with 21% receiving ceftazidime alone or in combination with another antibiotic. Previous exposure to antibiotics, especially third-generation cephalosporins, is commonly reported to be a risk factor for ESBL selection.[16]

More than 44% of our patients positive for ESBL-producing bacteria were from the hospital long-term care ward, and had indwelling urinary catheters. As asymptomatic bacteriuria is common among residents of long-term–care facilities with chronic indwelling urinary catheters,[17] antibiotics should only be given to patients with clinical evidence of infection to avoid the emergence of antibiotic-resistant organisms.[18] Of all the antibiotics we tested, carbapenems and amikacin demonstrated the highest activity against the ESBL-producing isolates. Our results showed differences in susceptibility patterns between *E. coli* and *K.*

pneumoniae for all the antibiotics excluding carbapenems and amikacin. The *K. pneumoniae* had lower susceptibility rates for gentamicin and higher susceptibility for piperacillin/tazobactam, cefepime and ciprofloxacin. A recent study from Taiwan has showed lower susceptibility rates to amikacin in *Klebsiella spp* than *E. coli*.[19] Another study from the United States showed ESBL-producing *E. coli* to have lower susceptibility rates to amikacin and gentamicin than *K. pneumoniae*.[20]

More than 12% of the ESBL-producing isolates were from outpatients, which indicate that infections with these organisms are not only confined to hospitals, but can also be acquired in the community. Previous antibiotic therapy was noted in 4 (15%) of the 26 outpatients positive for ESBL isolates, with ciprofloxacin most commonly received. Two (7.7%) of these patients had 2 or more significant risk factors, including urethral instrumentation. Hence, we recommend epidemiological monitoring for ESBL-producers among gram-negative bacteria causing urinary tract infections in both hospital and the community. Failure to recognize ESBL producing strains may result not only in inappropriate antimicrobial therapy and consequent therapy failure but also have infection control implications. The identification of ESBL-producing organisms is particularly important in developing countries where there is excessive use of antimicrobial drugs.

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