Prevalence of extended spectrum β-lactamase among multidrug resistant gram-negative isolates from a general hospital in Saudi Arabia

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ABSTRACT

Objective: To determine the prevalence of extended spectrum β-lactamase (ESBL) among multidrug resistant isolates of enterobacteriaceae and non-fermenting gram-negative bacilli.

Methods: This study was carried out at the Almana General Hospital, Eastern Province, Kingdom of Saudi Arabia, during the period March 2002 through to June 2003. Multidrug resistant gram-negative isolates from patients admitted to the surgical, medical, pediatric, long-term care and intensive care units were studied for the presence of the ESBL enzyme.

Results: A total of 3231 gram-negative organisms were studied for the presence of multidrug resistance and ESBLs. Of these, 197 (6%) isolates were multidrug resistant (MDR), and 156 (4.8%) were positive for ESBL. Seventy nine percent of the MDR strains were positive for ESBL. The most frequent isolates were *Escherichia coli* (1116) and *Klebsiella pneumoniae* (687) and ESBL was detected in 72 (6.5%) and 37 (5.4%) of these isolates. The MDR strains that produced ESBL were most commonly isolated from surgical care patients with diabetic fascitis (83%) and patients with indwelling Foley's catheter (79%). Extended spectrm β-lactamase producing strains showed the highest susceptibility to imipenem and meropenem (86%). The non-β-lactam antibiotics with greatest activity against these ESBL strains in vitro were ciprofloxacin (72%), amikacin (70%), tobramycin (67%) and gentamicin (56%).

Conclusion: The majority (79%) of the MDR enterobacteriaceae and non-fermenting gram-negative bacilli tested over 15-months were positive for ESBL. Imipenem, meropenem, ciprofloxacin and amikacin showed the highest activity against these ESBL-producing organisms. Due to the growing problem of infection with ESBL-producing bacteria, which are frequently resistant to many classes of antibiotics resulting in difficult-to-treat infections, clinicians need to be familiar with the clinical significance of these enzymes and potential strategies for dealing with them.

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Organisms producing extended-spectrum β-lactamases (ESBLs) have important therapeutic implications as they exhibit resistance to various antimicrobial agents, including third-generation cephalosporins, extended-spectrum penicillins, and monobactams.[1] The ESBL producing strains have variable susceptibility rates for fluoroquinolones, aminoglycosides, and fourth-generation cephalosporins.[2] The carbapenems are the only class of antibiotics uniformly active against ESBLs.[1] The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years resulting in limitation of therapeutic options.[3] Extended spectrum β-lactamase was first observed in 1983 in isolates of *Klebsiella pneumoniae (K.pneumoniae)* and Serratia species that had transferable plasmids encoding a mutated enzyme that made the bacteria resistant to

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cefotaxime.[4] Since then, these enzymes have been described in isolates of *Escherichia coli (E.coli)* and recently among *Salmonella species*.[5]

The ESBLs are derived from simple β-lactamase (TEM or SHV) enzymes. Selective pressure by the use of third generation cephalosporins favors the development of mutations that result in conformational changes (included in the active site) of amino acid sequence of the TEM or SHV enzymes.[6] Various risk factors for infection with ESBL strains have been identified including recent surgery, instrumentation, prolonged hospital stay and admission to intensive care unit.[7] The frequency of ESBL producing organisms differs significantly by geographic location. Despite the difficulties in laboratory detection of ESBL, it is important to identify the prevalence of ESBL production among members of Enterobacteriaceae in regional hospitals.

This study was carried out to determine the prevalence of ESBLs among the multidrug resistant gram-negative isolates from patients at Almana General Hospital, Eastern Province, Kingdom of Saudi Arabia over a period of 16-months.

Methods. This study was conducted in the Department of Clinical Microbiology of the Almana General Hospital. The period of the study extended from March 2002 through to June 2003. The 197 MDR isolates included in this study were from patients admitted to surgical, medical, pediatric, long-term care or intensive care units. One hundred and twenty-six patients were males and 71 were females. The average age was 59-years (range 1-93 years). The 197 isolates of MDR *E.coli*, *K. pneumoniae* and other gram-negative bacteria were tested for ESBL. The organisms positive for ESBL were isolated from clean catch midstream or catheter specimens of urine (84), wound swabs (61), sputum (5) and blood culture (6). Isolates were identified by standard microbiological methods.[8] The Kirby-Bauer disc diffusion method using Mueller Hinton agar and commercial antibiotic discs (Oxoid Limited, Hampshire, United Kingdom) were used for antimicrobial susceptibility testing. The following antibiotic discs were used, amoxycillin (10 mcg), amoxycillin + clavulanic acid (20/10 mcg), cephalexin (30 mcg), cefuroxime (30 mcg), cefotaxime (30 mcg), ceftazidime (30 mcg), cefepime (30 mcg), aztreonam (30 mcg), gentamicin (10 mcg), amikacin (30mcg), nitrofurantoin (300 mcg), trimethoprim (5 mcg), ciprofloxacin (5 mcg), imipenem (10mcg), meropenem (10 mcg), piperacillin (100 mcg) and piperacillin + tazobactam (100/10 mcg). The antibiotic disc impregnated culture plates were incubated at 37°C for overnight. The diameter of the zone of inhibition was measured and recorded as resistant or susceptible according to the National Committee for Clinical Laboratory Standards (NCCLS) interpretative criteria.[9] One hundred and ninety-seven MDR isolates showing resistance to third generation cephalosporins viz. cefotaxime and ceftazidime were tested for ESBL production. Minimum Inhibitory Concentration (MIC) against cefotaxime, ceftazidime and cefepime was carried out by agar dilution method for all isolates of *E.coli* and *K.pneumoniae* positive for ESBL. Testing for ESBL production was carried out using Mueller Hinton agar plates that were inoculated with standardized inoculum conforming to 0.5 McFarland standards of the suspected ESBL strain (as screened by the Kirby-Bauer disc diffusion technique)[10] to form a lawn culture. Separate commercial discs containing cefotaxime (30 mcg) and ceftazidime (30 mcg) with and without clavulanic acid (10 mcg) (Mast Diagnostics, Merseyside, United Kingdom) were placed over the lawn culture. A distance of 15 mm between the discs was maintained. An increase in zone size of more than or equal to 5 mm for cefotaxime and ceftazidime with and without clavulanic acid was considered to indicate ESBL producing strain. Control strains of *E.coli* (ATCC 25922) as a negative control and *K.pneumoniae* (ATCC 700603) as a positive control were included.[9]

Results. Of the 197 MDR gram-negative bacilli tested, 156 (79%) were found to be positive for ESBL. The number positive from Enterobacteriaceae family was 136 (87%); and from non-fermenting gram-negative bacilli 20 (12.8%). The ratio between the 2 was 6.8-1. Sixty-one isolates from wound specimens were ESBL producers; the most frequent organisms were *E.coli* (44%) and *K.pneumoniae* (24.6%), **Table 1**. Eighty-four urinary isolates from patients with urinary tract infection (UTI) were ESBL producer. Of these, 71 were catheter specimen urine from patients with indwelling Foley's catheter, 15 following a catheter insertion and 24 following urological procedures (ureteric stenting), **Table 2**. The most frequent isolate was *E.coli* (50%) followed by *K. pneumoniae* (21%), **Table 1**. Four isolates of Acinetobacter species from patients who had chronic obstructive pulmonary disease (COPD) were positive for ESBL. One isolate of *E.coli* from a patient with bilateral lower lobe pneumonia was positive for ESBL **(Table 1)**. The blood culture of 6 patients with septicemia grew multidrug resistant gram-negative organisms; *E.coli* (2) and *K. pneumoniae* (4). They were all positive for ESBL **(Table 1)**. The clinical diagnosis and or infection for patients with isolates positive for ESBL are listed in **Table 2**.

More than 95% of the ESBL producing *E.coli* showed an MIC of >-256 mcg/ml against cefotaxime, ceftazidime and cefepime. More than

Table 1 - Number and percentage of extended spectrum β-lactamase producing organisms from specimens positive for extended spectrum β-lactamase.

Specimens	*E.coli*	*K.pneumoniae*	*Enterobacter sp.*	*Citrobacter sp.*	*P.aeruginosa*	*Acinetobacter sp*	Total
Urine	42 (50)	18 (21)	12 (14.3)	6 (7.1)	4 (4.8)	2 (2.4)	**84**
Wound stab	27 (44)	15 (24.6)	6 (9.8)	3 (4.9)	3 (4.9)	7 (11.5)	**61**
Sputum	1 (20)	-	-	-	-	4 (80)	**5**
Blood	2 (33)	4 (66.7)	-	-	-	-	**6**
Total	**72 (46)**	**37 (21.6)**	**18 (11.5)**	**9 (5.8)**	**7 (4.5)**	**13 (8.3)**	**156**

E.coli - Escherichia coli, P - pseudomonas, K - klebsiella, sp - species

Table 2 - Number and percentage distribution of extended spectrum β-lactamase.

Diagnosis	n of MDR gram-negative isolates	n of ESBL strains	Percentage of ESBL strains
UTI following indewelling Foley's catheter	71	56	79
Post operative UTI following catheter insertion	15	9	60
UTI following ureteric stenting	24	19	79
Diabetic fascitis	46	38	83
Open wounds (traumatic and open ulcers)	17	14	82
Post operative wound	9	6	67
Burn wound	3	3	100
COPD	4	4	100
Pneumonia	2	1	50
Septicemia	6	6	100
Total	**197**	**156**	**79**

UTI - urinary tract infection
COPD - chronic obstructive pulmonary disease

97% of the ESBL producing *K.pneumoniae* showed an MIC of >256 mcg/ml against cefotaxime, ceftazidime and cefepime **(Table 3)**. Extended spectrum β-lactamase producing isolates showed the highest in vitro susceptibility to imipenem and meropenem (85.6%). The following non-beta lactam antibiotics showed the greatest in vitro activity against the ESBL strains: ciprofloxacin (72%), amikacin (70%), tobramycin (67%) and gentamicin (56%), **Table 4**.

Discussion. The majority (85.2%) of our ESBL isolates were from patients under long term care followed by ICU (80.9%). Among the different clinical samples, ESBL producers were found most commonly in isolates from cases of diabetic fascitis (83%) and UTI (79%) associated with indwelling Foley's catheter and ureteric stenting **(Table 2)**. The length of hospital stay, and urinary catheterization are important risk factors for acquisition of ESBLs.[11] Among the multidrug resistant isolates we studied, E. coli was the highest to produce ESBL (46%), followed by *K.pneumoniae* (21.6%). Many past studies had shown ESBL producing K. pneumoniae to be more prevalent than ESBL producing *E.coli*.[7,12-14]

In a previous study carried out in Abha, KSA,[15] extended-spectrum β-lactamase (ESBL) was detected in 27.5% of ceftazidime-resistant *K. pneumoniae* isolated in 1999, which is slightly higher than ours (21.6%). The prevalence of ESBL in our gram-negative isolates was more than 20% higher than those reported in some Indian studies[16,17] in which 53% and 56.1% of their multidrug resistant gram-negative isolates were ESBL producers. Imipenem and meropenem showed the highest activity against the ESBL producing isolates (85.6%). The non-β lactam antibiotics with greatest activity against the ESBL producing strains in our study were ciprofloxacin (72%), followed by amikacin (70%), tobramycin (67%) and gentamicin (56%). These antibiotics could be used as effective alternatives in the treatment of patients infected with ESBL strains. Ninety-one (58%) patients with positive ESBL isolates had received repeated courses of antibiotics (particularly ceftazidime), which could have contributed to the high incidence of ESBL producing organisms among these patients. Hospitals should have proper policies and guidelines for the prudent use of antimicrobial agents in order to reduce or discourage the emergence and spread of ESBL producing organisms. Since these ESBL strains develop resistance to various antibiotics and can spread rapidly, appropriate and efficient infection control measures should be followed and practiced by the

Table 3 - Minimum inhibitory concentration of extended spectrum β-lactamase producing strains of *Escherichia coli* and *Klebsiella pneumoniae*.

ESBL positive bacteria	Total number	Cefotaxime	Ceftazidime	Cefepime
E.coli	**72**	≥256 mg/ml (69) 128 mg/ml (3)	≥256 mg/ml (70) 128 mg/ml (2)	≥256 mg/ml (70) 128 mg/ml (2)
K.pneumoniae	**37**	≥256 mg/ml (36) 128 mg/ml (1)	≥256 mg/ml (36) 128 mg/ml (1)	≥256 mg/ml (37)

ESBL - extended spectrum β-lactamase, E - *Escherichia,* K - *Klebsiella*
Number within parenthesis indicates number of isolates

Table 4 - Number and percentage of non-β-lactam antibiotics active against extended spectrum β-lactamase producing organisms.

Organisms	IMP	MEP	CIP	AK	GN	TOB
E.coli (72)	62 (86)	62 (86)	52 (72)	50 (69)	41 (57)	48 (67)
K.pneumoniae (37)	32 (86)	32 (86)	27 (73)	26 (70)	21 (58)	25 (68)
Enterobacter sp (18)	15 (83)	15 (83)	13 (72)	13 (72)	10 (56)	12 (67)
Citrobacter sp (9)	8 (89)	8 (89)	7 (76)	6 (67)	5 (56)	6 (67)
P.aeruginosa (7)	6 (86)	6 (86)	5 (71)	5 (71)	4 (57)	5 (71)
Acinetobacter sp (13)	11 (85)	11 (85)	9 (69)	9 (69)	7 (54)	9 (69)
Total	**134 (11)**	**134 (86)**	**113 (72)**	**109 (70)**	**88 (56)**	**105 (67)**

IMP - imepenem, MEP - meropenem, CIP - ciprofloxacin, AK - amikacin, GN- gentamicin, TOB - tobramycin
Number within parenthesis against the anitbiotics indicates percentages
Number within parenthesis besides the organisms indicates number of isolates
E - Escherichia, K - Klebsiella, P - pseudomonas

healthcare staff involved in patient care. Many clinicians would be unaware of ESBL producing isolates if microbiology laboratories did not report them. In addition, these resistant organisms may escape detection with routine susceptibility testing carried out by a clinical microbiology laboratory, which can result in treatment failures.[18] Therefore, detection of ESBL strains in the laboratory becomes necessary. National Committee for Clinical Laboratory Standards[9] criteria can be used to detect ESBL. Clinical microbiology laboratories must adopt a technique most suitable to them for ESBL detection. Data regarding the prevalence of ESBL strains in various hospitals from KSA is limited.[19] It must also be emphasized that extrapolation of the results of studies conducted abroad will be highly unrepresentative to the present Saudi Arabian situation. The level of information regarding the prevalence of ESBL can be increased by conducting studies in local hospitals in KSA, thereby providing important data for clinicians and also for comparing the prevalence of ESBL producing organisms at both local and national levels.

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