Prevalence of extended-spectrum beta-lactamases-producing isolates over a 1-year period at a University Hospital in Oman

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ABSTRACT

Objective: To evaluate the prevalence of extended-spectrum ß-lactamases isolates over one year period at Sultan Qaboos University Hospital.

Methods: We identified the ESBL isolates during a 12-month period from July 2004 to June 2005, using a commercial system, and confirmed the result using the National Committee for Clinical Laboratory Standards-approved double-disk diffusion method.

Results: Sensitivity was recorded for a wide range of antibiotics, aminoglycosides, carbapenem, cephalosporins, quinolones, aztreonam, ampicillin, amoxicillin/ clavulanate, ampicillin/sulbactam, piperacillin-tazobactam, trimethoprim/ sulfamethoxazole and nitrofurantoin. Of the total ESBL isolated, 29.6% were from medical ward, followed by outpatients clinic, 24.3%. Urine was the main source of ESBLs 70.4%, followed by 16.5% from blood. We observed a 100% sensitivity to carbapenems, whereas 93.9% of the isolates were susceptible to amikacin. Cephalosporins were 100% resistant, except for cefoxitin, which demonstrated sensitivity of 77.4%. Aztreonam, ampicillin, co-amoxyclav and ampicillin/sulbactam were 100% resistant. Of the isolates, 57.4% were sensitive to nitrofurantoin, whereas Tazocin showed 49.6% sensitivity and co-trimoxazole 13.9%. To quinolones, 74.8% of the isolates were resistant.

Conclusion: Excess use of third generation cephalosporins led to increase rate of ESBLs, which are difficult to treat. Carbapenem are most reliable for treatment of infections caused by ESBL isolates. However, overuse of carbapenem may lead to resistance of other gram-negative organisms. Therefore, justifiable use of third-generation cephalosporins, will be an effective means of controlling and decreasing the spread of ESBL isolates.

Saudi Med J 2007; Vol. 28 (1): 22-27

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Received 26th March 2006. Accepted 6th September 2006.

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Extended-spectrum β-lactamase (ESBL) producing isolates are a growing problem worldwide for clinicians dealing with infectious disease.[1,2] The first ESBL isolates were discovered in Western Europe in the mid-1980s and subsequently in the United States in the late 1980s.[3-5] The ESBL producing organisms are among the fastest growing problems in the area of infectious diseases. These β-lactamases can be produced by a variety of *Enterobacteriaceae*; however, the most common ESBL-producing organisms are *Klebsiella pneumoniae (K. pneumoniae),* other *Klebsiella* species (namely, *Klebsiella oxytoca*), and *Escherichia coli (E. coli).*[6] Other organisms reported to harbor ESBLs include *Enterobacter* species, *Salmonella enterica, Morganella morganii, Proteus mirabilis, Serratia marcescens,* and *Pseudomonas aeruginosa.* However, the frequency of ESBL production in these organisms are low.[7-10]

With the ability to produce highly effective β-lactamase enzymes, these organisms are resistant to all β-lactam antibiotics except cephamycins (cefoxitin, cefotetan) and carbapenems. Most ESBL enzymes contain a serine at the active site and belong to Ambler's molecular class A,[11] a classification scheme devised by Bush et al.[12] The ESBLs are β-lactamases that hydrolyze extended-spectrum cephalosporins with an oxyimino side chain.[13] These cephalosporins include cefotaxime, ceftriaxone, and ceftazidime, as well as the oxyimino-monobactam aztreonam. The clinical relevance of ESBLs has been well documented by numerous published case reports describing clinical failures with the use of third-generation cephalosporins such as these oxyimino-cephalosporins (cefotaxime, ceftriaxone, ceftazidime) as well as with the use of cefoxitin and the fourth-generation cephalosporin, cefepime.[14] Thus, the problem of ESBLs is clinically important, yet remains relatively unappreciated by most clinicians.[15,16] This is because many clinical microbiology laboratories continue to mistakenly report these Gram-negative bacillary isolates as

susceptible due to difficulties in identifying isolates, which possess these important beta-lactamases.[14,17,18] However, a few important points should be remembered when comparing ESBL producing organisms with other β-lactamases producing organisms. Traditional TEM and SHV β-lactamases can be inhibited by β-lactam-β-lactamase inhibitor combinations and extended-spectrum cephalosporins (such as, ceftazidime, cefotaxime, and ceftriaxone).

As of 25 January 2005, there were 138 TEM- (TEM-1 to TEM-139) and 62 SHV-type of (SHV-1 to SHV-63) β-lactamases, mostly found in *K. pneumoniae* and *E. coli* strains.[19] In addition, ESBL-producing organisms are frequently resistant to many other classes of antibiotics, including fluoroquinolones the monobactam aztreonam, while resistance to trimethoprim–sulfamethoxazole and aminoglycosides is frequently co-transferred on the same plasmid.[19-23]

An ESBL-producing organisms differ significantly from AmpC β-lactamase-producing organisms as ESBL producers are generally susceptible to cephamycins (such as cefoxitin) in vitro, and genes on plasmids encode ESBLs. The location of these genes on the plasmids results in easier transfer of ESBL enzymes to other bacterial species compared with AmpC β-lactamase enzymes, which are located on the chromosomes of *Enterobacter* species, *Citrobacter freundii, Morganella morganii, Serratia marcescens,* and *Pseudomonas aeruginosa.* However, reports of plasmid-mediated AmpC enzymes will further the difficulties of phenotypically identifying ß-lactamases.[24-29]

The National Committee for Clinical Laboratory Standards (NCCLS) recommends that microbiology laboratories report ESBL-producing isolates of *E. coli* and *Klebsiella* species as resistant to all penicillins, cephalosporins (including cefepime), and aztreonam irrespective of their individual in vitro test results.[30] The presence of ESBLs in some *K. pneumoniae* and *E. coli* strains poses an important challenge in clinical practice, since these organisms are common causes of serious infections. Imipenem and meropenem are considered the therapy of choice for patients with serious infections due to ESBL producing strains.[19]

Many ESBL-producing isolates are not always phenotypically resistant to oximino-cephalosporins. However, patients suffering from infections caused by ESBL-producing organisms are at risk of treatment failure if an extended spectrum of cephalosporins (ESC) are prescribed.[19,31,32] Therefore, it is imperative for the clinical microbiology laboratory to identify isolates that possess increased MICs (≥2 μg⁄mL) to oximino-cephalosporins, even though they may be equal to or below the susceptibility breakpoint (MIC ≤8 μg⁄mL).[19]

Retrospective study at Sultan Qaboos University Hospital (SQUH) demonstrated an alarming increase in the prevalence of ceftazidime-resistant *K. pneumoniae* during a 6-year period from 4% in 1999 to 9% in 2004. *Escherichia coli* showed an increase in resistance to ceftazidime from 3% in 1999 to 6% in 2004, whereas *Klebsiella species* showed an increase in resistance to Ceftazidime from 2% in 1999 to 10 % in 2004 (**Table 1**). This led us to look into our antibiotic resistance problems that maybe being caused by the ESBLs with our inpatients as well as those attending the outpatient clinic at SQUH.

Methods. Sultan Qaboos University Hospital is a 500-bed, International Organization for Standardization - Certified Teaching Hospital located on the campus of Sultan Qaboos University in Muscat, Oman. The institution is the leading referral civilian Hospital in Oman, with physicians representing all major medical specialties.

Microbiologic analysis. Specimens processed by the department of microbiology during the 12-month period from July 2004 to June 2005 that yielded a confirmed ESBL isolate were included in this study. Isolates were screened initially for ESBL production by a commercial system ("Phoenix Identification and susceptibility system" by Becton Dickinson). All ESBL isolates were confirmed using the Clinical and Laboratory Standards Institute-approved double-disk diffusion method.[23] A positive result required an increased zone (≥5 mm), using ceftazidime or cefotaxime disks combined with clavulanate compared with either drug alone.[19,33,34] Susceptibility data were recorded for the following antimicrobials using the phoenix: gentamicin, amikacin, tobramycin, imipenem, meropenem, 4 generation of cephalosporins: cefazoline, cephalothin, cefuroxime sodium, cefoxitin, ceftazidime, cefotaxime and cefepime, aztreonam, ampicillin, amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin-tazobactam, trimethoprim/sulfamethoxazole, nitrofurantoin, ciprofloxacin, norfloxacin and nalidixic acid were tested.

Results. A total of 115 ESBL-producing isolates were obtained over a one-year study period from out and in-

Table 1 - Changing resistance pattern at Sultan Qaboos University Hospital for ceftazidime between 1999-2004.

Isolates	1999	2000	2001	2002	2003	2004
	Ceftazidime resistance %					
Escherichea coli	(3)	(2)	(3)	(6)	(7)	(6)
Klebsiella pneumoniae	(4)	(2)	(2)	(7)	(6)	(9)

patients who attended the SQUH, 68 were females and 47 were male patients. Out of 115 ESBLs, 60% were *E. coli* and 40% were *K. pneumoniae*. Majority (29.6%) of these ESBLs were from the medical wards followed by patients who attended the outpatients clinic at SQUH (24.3%). Accident and Emergency and Surgical Units contributed 10.4% each, followed by Intensive Care Unit (9.6%). The least number of ESBLs isolated were from the children wards (7%), neonatology (5.2%) and obstetric and gynecology (3.5%) (**Table 2**). Urine (70.4%) was the main source of ESBLs from all patients. Of the ESBLs, 16.5% were from blood sample, tracheal aspirates/sputum contributed to 8.7% followed by 3.5% from swabs and 0.9% fluids (**Table 3**).

Microbiologic analysis. Susceptibility data are reported in **Table 4**, several trends of particular interest were observed. All isolates were susceptible to both imipenem and meropenem. Of all isolates (93.9%) were susceptible to amikacin, whereas only 31.3% was susceptible to gentamicin and 20% to tobramycin. The cephalosporins (1-4 generations) were all (100%) resistance, although they may have demonstrated a low MIC, but the final interpretation was resistant, except for cefoxitin, which demonstrated a sensitivity of 77.4% against all isolates. For nitrofurantoin, 57.4% were sensitive. Tazocin showed a sensitivity of 49.6%, whereas low sensitivity was shown towards co-trimoxazole 13%. Ciprofloxacin and norfloxacin were 23.5% sensitive whereas only 17.4% were sensitive to nalidixic acid (**Table 4**). High resistance of 74.8% was observed to all quinolones (ciprofloxacin norfloxacin and nalidixic acid) tested, whereas only 5.2% of the isolates were resistant to all aminoglycosides tested (**Table 5**). Aztreonam, ampicillin, co-amoxyclav and ampicillin/sulbactam were 100% resistant. Compared to other antibiotics both *E. coli* and *K. pneumoniae* were more sensitive to cefoxitin and amikacin (**Table 4**). Quinolones sensitivity was greater for *K. pneumoniae*, than *E. coli*, whereas nitrofurantoin and Tazocin sensitivity was greater for *E. coli*. There was no difference in sensitivity to cefoxitin or amikacin, for *E. coli* or *K. pneumoniae,* 100% sensitivity was observed for both imipenem and meropenem.

Imipenem, meropenem, cefoxitin and amikacin sensitivity were greater for blood and urine isolates (**Table 4**). Nitrofurantoin and cefoxitin showed greater sensitivity for respiratory isolates, but quinolones were 100% resistant to respiratory and swab samples (**Table 4**). Isolates from the neonatology wards were more sensitive compared with other wards (**Table 4**).

Discussion. The results of this study demonstrate that ESBL-producing organisms continue to be a problematic pathogens. Our study demonstrated clear differences in susceptibility patterns with our 115 ESBLs, between *K. pneumoniae* and *E. coli* for amikacin, gentamicin, trimethoprim-sulfamethoxazole, Tazocin, nitrofurantoin, fluoroquinolones and cefoxitin. Studies at other centers reported susceptibility patterns similar to our results at SQUH, for some antimicrobials; however, none of the studies have provided patterns identical to those of our study,[14,35-37] This is probably because the ESBL is located on a plasmid that can be transferred from one organism to another rather easily and can incorporate genetic material coding for resistance to other antimicrobial classes.

Majority (29.6%) of the ESBLs were from medical wards followed by patients who attended the outpatients clinic at the SQUH (24.3%) (**Table 2**). The high percentage of ESBLs from outpatient clinics and accident and emergency should alert the physician in the primary care regarding the complication of uncontrolled prescription of oral cephalosporin, such as, cefuroxime. It should also alert them regarding the probability of ESBLs in infections not responding to the first line antibiotics, such as amoxicillin. Urine (70.4%) was the main source of ESBLs from all patients, followed

Table 2 - Frequency and percentage of extended-spectrum ß-lactamases from different wards at the Sultan Qaboos University Hospital.

Wards	Frequency	(%)
Accident and emergency	12	(10.4)
Children's ward	8	(7)
Intensive care unit	11	(9.6)
Medical ward	34	(29.6)
Neonatology	6	(5.2)
Obstetric and gynecology	4	(3.5)
Outpatient clinic	28	(24.3)
Surgical wards	12	(10.4)

Table 3 - Frequency and percentage of extended-spectrum ß-lactamases from different source at the Sultan Qaboos University Hospital.

Source	Frequency	(%)
Blood	19	(16.5)
Fluid	1	(0.9)
Swabs	4	(3.5)
Tracheal aspirates/ Sputum	10	(8.7)
Urine	81	(70.4)

by blood (16.5%) (**Table 4**). Generally, ESBLs isolated from medical wards and from blood appeared to be more sensitive than isolates from other areas and source (**Table 4**). Respiratory and other swabs samples did not demonstrate any sensitivity for all the quinolones and trimethoprim-sulfamethiazole tested. Tobramycin demonstrated a 100% resistance towards all isolates (**Table 4**).

Apart from carbapenems, which was 100% sensitive, amikacin was the next most sensitive of all antibiotics tested (93.9%) followed by cefoxitin (77.4%) (**Table 4**). In our study ciprofloxacin, norfloxacin and nalidixic acid showed significant differences in susceptibility for all isolates. Though *Klebsiella pneumoniae* were more susceptible than *E. coli* toward quinolones, the overall resistance for all quinolones was as high as 74.8%, which was a significant observation (**Table 5**). This correlates well with a recent study from Taiwan,[38] which makes quinolones a bad choice for gram-negative sepsis not responding to third generation cephalosporins.

Gentamicin and amikacin susceptibility was greater in *E. coli* than *K. pneumoniae* (**Table 4**), which also correlates well with results from Taiwan.[38] Only 5.2% of all isolates were resistant to amikacin, gentamicin and tobramycin together, this was significantly less than

Table 4 - Susceptibility patterns of extended-spectrum ß-lactamases organisms isolated fro Sultan Qaboos University Hospital July 2004-July 2005.

Variable	Percentage of susceptible isolates										
	Penem	Cefox	Ak	Cn	Tobra	Taz	Nitro	Cip	Nor	Na	Sxt
Isolates											
All	100	77.4	93.9	31.3	20	49.6	57.4	23.5	23.5	17.4	13.9
Escherichea. coli	100	79.7	94.2	36.2	17.4	56.5	88.4	15.9	13	7.2	15.9
Klebsiella pneumoniae	100	73.9	93.5	23.9	23.9	39.1	10.9	34.8	39.1	32.6	10.9
Source											
Urine	100	75.3	95.1	28.4	16	43.2	55.6	21	22.2	13.6	18.5
Blood	100	89.5	100	57.9	47.4	68.4	52.6	47.4	42.1	42.1	0
Respiratory	100	80	70	10	0	60	90	0	0	0	0
Swabs	100	50	100	0	0	75	50	0	0	0	0
Wards											
Medical	100	67.6	97.1	26.5	14.7	47.1	70.6	14.7	17.6	8.8	5.9
Outpatient clinic	100	85.7	89.3	14.3	10.7	46.4	53.6	14.3	14.3	7.1	17.9
Intensive care unit	100	81.8	90.9	27.3	18.2	72.7	81.8	27.3	27.3	27.3	9.1
Neonatology	100	100	100	100	100	100	0	100	100	100	0
Accident and emergency	100	83.3	100	50	16.7	58.3	50	41.7	33.3	16.7	25
Surgery	100	66.7	91.7	16.7	16.7	16.7	50	8.3	8.3	8.3	25
Children	100	75	87.5	62.5	37.5	62.5	62.5	25	25	25	12.5
OBS/GYN	100	75	100	25	0	0	25	25	25	25	25

Penem – carbapenem, Cefox - cefoxitin, Ak – Amikacin, CN – gentamicin, Tobra – tobramycin, Taz - piperacillin-tazobactam, Nitro – nitrofurantoin, Cip – ciprofloxacin, Nor – Norfloxacin, NA - nalidixic acid, SXT - trimethoprim- sulfamethoxazole

Table 5 - Resistance to one or more aminoglycosides or quinolones of 115 extended-spectrum ß-lactamases.

Extended-spectrum beta lactamases	Frequency	Percentage
Aminoglycosides		
Resistant to amikacin, gentamicin and tobramycin	6	(5.2)
Resistant to gentamicin and tobramycin	73	(63.5)
Resistant to amikacin and tobramycin	1	(0.9)
Resistant to tobramycin	12	(10)
Sensitive to amikacin, gentamicin and tobramycin	23	(20)
Quinolones		
Resistant to ciprofloxacin, norfloxacin and nalidixic acid	86	(74.8)
Resistant to ciprofloxacin and nalidixic acid	2	(1.7)
Resistant to norfloxacin and nalidixic acid	2	(1.7)
Resistant nalidixic acid	5	(4.3)
Sensitive to ciprofloxacin, norfloxacin and nalidixic acid	20	(17.4)

that for the quinolones (**Table 5**). Cefoxitin sensitivity was also high for both *E. coli* and *K. pneumoniae* (**Table 4**). High sensitivity of ESBLs to amikacin makes it a possible candidate for synergy with carbapenem in severe ESBL sepsis. The susceptibility patterns for piperacillin-tazobactam against ESBL organisms have been extremely variable. Some investigators have reported susceptibility rates for ESBL pathogens greater than 90%, whereas others have seen large differences based on organisms.[14,35-37] In our study at SQUH, there was a significant difference in piperacillin-tazobactam susceptibility between *E. coli* and *K. pneumoniae* (**Table 4**). *Escherichia coli* (56.9%) showed higher susceptibility than *K. pneumoniae* (39.1%). This percentage susceptible was much lower to that reported in one of the study.[15]

Overall prevalence of ESBL-producing isolates at our institution was high compared to reports from United States and Europe.[15] Given the very limited treatment options available for these pathogens, prevention remains a significant priority in controlling the development and spread of ESBL organisms. Several studies have demonstrated that a modifiable risk factor for the development of ESBL-producing organisms is the use of third-generation cephalosporins.[23-27] Hence, formulary modification at SQUH by decreasing the use of third-generation cephalosporins and increasing the use of imipenem, meropenem with amikacin or piperacillin-tazobactam should significantly decrease in the isolation of ESBL-producing bacteria.[14,40-45] However, increased carbapenem administration has been associated with increases in carbapenem-resistant *Acinetobacter* species and carbapenem-resistant *P. aeruginosa,* while increased piperacillin-tazobactam administration has not been associated with significant increases in organisms resistant to piperacillin-tazobactam.[40,43-44] Although there are conflicting arguments regarding the use of piperacillin-tazobactam in treatment of ESBLs, it has been demonstrated to be sensitive in vitro.[19] Wong-Beringer[41] suggested the use of piperacillin–tazobactam in the case of a non-outbreak situation, to preserve the therapeutic value of carbapenem.

The ESBL-producing organisms are increasing rapidly and becoming a major problem in the area of infectious diseases. In one study from Turkey the prevalence rate of ESBLs was 12-47%.[45] High rates of third-generation cephalosporin use have been implicated as a major cause of this problem.[23-27] Problems associated with ESBLs include multidrug resistance, difficulty in detection and treatment, and increased mortality. Of all available antimicrobial agents, carbapenem are the most sensitive and reliable treatment options for infections caused by ESBL isolates. However, overuse of carbapenem may lead to resistance of other gram-negative organisms. Therefore, restricting the use of third-generation cephalosporins, along with implementation of infection control measures, are the most effective means of controlling and decreasing the spread of ESBL isolates.

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