

Extended spectrum beta-lactamases (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: trends in the hospital and community settings

Husam S. Khanfar^{1,2*}, Khalid M. Bindayna^{2*}, Abiola C. Senok³, and Giuseppe A. Botta⁴

¹Department of Microbiology and Epidemiology, Dhahran Health Centre, Saudi Aramco Company, Eastern Province, Saudi Arabia

²Department Microbiology, Immunology and Infectious diseases, Arabian Gulf University Manama Bahrain

³Department of Clinical Sciences, College of Medicine, University of Sharjah, United Arab Emirates

⁴Microbiology section Dept Medical and Morphological Sciences, Udine Medical School, Italy

*These authors contributed equally to this work

Abstract

Background: To assess the prevalence of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* strains in nosocomial and community-acquired infections.

Methodology: The study was conducted at a centralized microbiology laboratory in the Eastern Province of Saudi Arabia. Laboratory records (January 2004- December 2005) were assessed. Associated resistance to a panel of antibiotics was determined.

Results: A total of 6,750 Gram-negative organisms were assessed for ESBL-phenotype. ESBL was detected in 6% (409/6,750) of isolates, the majority of which were *E. coli* (83%). ESBL producers were significantly higher among isolates from in-patients 15.4% (143/927) versus out-patients (4.5%; 266/5,823); $p < 0.05$. Old age (older than 60 years) represented a significant risk for having an ESBL-producing pathogen. Urine was the major source of ESBL isolates in in-patients (46.1%) and out-patients (74.4%). The proportion of urinary *E. coli* isolates which were ESBL producers was significantly higher among in-patients (53/506; 10.4%) compared to out-patients (182/4,074; 4.4%); $p < 0.05$. Among in-patients, 60% of the ESBL associated infections were nosocomial. All were sensitive to imipenem but high levels of resistance to gentamicin, amikacin, amoxicillin-clavulanic acid and ciprofloxacin was shown.

Conclusion: The findings document evidence of the spread of multiresistant ESBL-producers into the community. This has significant implications for patient management, and indicates the need for increased surveillance and molecular characterization of these isolates.

Key words: extended spectrum beta lactamase, antibiotic resistance, *Escherichia coli*, *Klebsiella pneumoniae*

J Infect Dev Ctries 2009; 3(4):295-299.

Received 3 February 2009 - Accepted 4 April 2009

Copyright © 2009 Khanfar *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

In 1983, the first outbreak involving extended spectrum beta-lactamase (ESBL) producing organisms was reported in Germany [1]. These ESBL-producing pathogens are now recognized globally as major causes of nosocomial and community-acquired infections [2]. ESBLs arise because of mutations in the TEM-1, TEM-2, or SHV-1 genes, commonly found in the *Enterobacteriaceae* family [2,3]. While these enzymes are found predominantly in *Klebsiella* species and *Escherichia coli*, they have also been described in other genera of *Enterobacteriaceae* including *Citrobacter*, *Serratia*, *Proteus*, *Salmonella*, and *Enterobacter* [2]. They are capable of hydrolyzing penicillins, broad-spectrum cephalosporins and monobactams, but they do not affect the cephamycins or carbapenems and their activity is inhibited by clavulanic acid [3].

The prevalence of bacterial isolates expressing the ESBL phenotype varies across different geographical regions with low rates of 3-8% reported in Sweden, Japan and Singapore compared to much higher prevalence rates documented in studies from Portugal (34%), Italy (37%), New York (44%), Latin American countries (30-60%) and Turkey (58%) [3]. In the Arabian Peninsula, reported ESBL detection rates range from 8.5-38.5% in data from the Kingdom of Saudi Arabia [4-8] and (31.7%) in Kuwait [9]; the highest level of 41% is from the United Arab Emirates [10]. This variation is perhaps reflective of the fact that these studies focused on hospital-acquired infections or particular sites of infection (*e.g.* urine, blood). In this report, we present the first direct comparative data on the prevalence and characteristics of ESBL-producing *Enterobacteriaceae* causing hospital and community-acquired infections in Saudi Arabia.

Materials and Methods

Data collection

Retrospective analysis of laboratory records at the Saudi Aramco Dhahran Health Center, over a two-year period from January 2004 to December 2005, was conducted. The Centralized Microbiology Laboratory at this 405-bed facility also serves the 80-bed Al-Hasa hospital, as well as three out-patient clinics and an emergency medical service in the surrounding communities. Patients' demographic data, clinical diagnoses, and specimen types were recorded for all *E. coli* and *Klebsiella* strains isolated during the study period. The number of isolates found to be ESBL producers and their antibiotic susceptibility profiles were noted. Only one positive culture per patient was included in the study and repeated positive cultures from the same patient were excluded from the analysis. Isolates for which it was impossible to discriminate between contamination and infection were excluded from the analysis. Hospital associated infection was defined as occurrence of infection 48 hours or more after hospital admission, without evidence that the infection was present or incubating on admission, in patients without prior history of stay in a healthcare facility [11].

Detection of ESBL isolates

E. coli and *Klebsiella* isolates reported as cephalosporin resistant or ESBL using the automated VITEK Gram Negative Susceptibility System with cards GNS 206 and 121 (bioMérieux, Vitek Inc, Hazelwood, USA) were screened for ESBL detection using the disk diffusion method. In keeping with the Clinical and Laboratory Standards Institute (CLSI) recommended guidelines [12], ESBL screening was performed by disk diffusion using ceftazidime (10ug) cefpodoxime (30ug) and aztreonam (30ug) disks. Confirmation of ESBL phenotype was performed by the double disk diffusion method using antibiotic disks containing a combination of cephalosporin plus clavulanic acid in conjunction with a corresponding cephalosporin disk alone. The following antibiotic disks were used: ceftazidime (CAZ 30µg), ceftazidime plus clavulanic acid (CAZ/CA 30/10µg), cefotaxime (CTX 30µg) and cefotaxime plus clavulanic acid (CTX/CA 30/10µg). Antibiotics were all obtained from Becton Dickinson, USA. The tests were interpreted according to CLSI guidelines [12] and ≥ 5 mm increase in the zone of inhibition for the CAZ/CLA and CTX/CLA containing disk versus

the corresponding CAZ or CTX disk was considered positive for ESBL.

The antibiotic susceptibility pattern of the ESBL-producing isolates to a panel of antibiotics including amikacin, amoxicillin plus clavulanic acid, ciprofloxacin, gentamicin, imipenem, piperacillin and ticarcillin was recorded. *E. coli* ATCC 25922 was used to control for growth of isolates and potency of antibiotic disks. Data analysis (chi-squared test) was done using SigmaStat version 3.5 (Systat Software Inc, San Jose California, USA) and $p < 0.05$ was considered statistically significant.

Results

During the study period, 6,750 Gram negative organisms comprised of 5,503 (82%) *E. coli*; 1,180 (17%) *K. pneumoniae*; and 67 (1%) *K. oxytoca* were assessed for ESBL phenotype. ESBL was detected in 6% (409/6,750) of isolates with detection rate of 5.8% (190/3,232) in 2004, which increased to 6.2% (219/3,518) in 2005. The majority (83%) of the ESBL-producing isolates were *E. coli*. ESBL producers were significantly higher among isolates from in-patients 15.4% (143/927) compared to those from out-patients (4.5%; 266/5,823) ($p < 0.05$). Table 1 shows the distribution of ESBL isolates among the in-patient and out-patient groups. Among the isolates obtained from in-patients 21% (30/143) were from patients in the intensive care unit. The majority of the ESBL isolates (229/409; 56%) were identified in patients older than 60 years, followed by 31.5% (129/409) in those aged 21 to 60 years, 7.1% (29/409) from the 11- to 20-year-old group, and 5.4% (22/409) in those younger than 10 years. It is significant that, although 56% of ESBL isolates were identified in patients older than 60years, this age group constituted of only 16.2% (1,094/6,750) of the total number of patients from whom *E. coli* and *Klebsiella* were isolated during the study period.

Urine was the major source of ESBL-producing isolates in both in-patients (46.1%) and out-patients (74.4%) (Table 2). As the majority of the ESBL isolates in both patient populations were *E. coli* and of urinary source, we determined the proportion of urinary *E. coli* isolates which were ESBL producers in both patient populations. Among in-patients, 506 *E. coli* isolates came from urine specimens compared to 4,074 among out-patients. The proportion of urinary *E. coli* isolates which were ESBL producers was significantly higher among in-patients (53/506; 10.4%) compared to out-patients (182/4,074; 4.4%); $p < 0.05$. Among the 143 in-patients from whom

ESBL isolates were cultured, there was available data which enabled us to categorize whether the infection was nosocomial or community acquired in 126 patients (88%). Of these, 75 (60%) were nosocomial compared to 51 (40%) which were community acquired. The majority (74%) of *K. pneumoniae* infections were nosocomial in origin. In contrast, distribution of *E. coli* isolates in nosocomial and community-acquired infections were 54% and 45% respectively.

All ESBL-producing isolates were sensitive to imipenem. Resistance to a panel of antibiotics including gentamycin, amikacin, augmentin, ciprofloxacin, ticarcillin and piperacillin was demonstrated. All the ESBL *Klebsiella* isolates were resistant to both piperacillin and ticarcillin and only two *E. coli* isolates were sensitive to these two antibiotics. The proportions of ESBL producing *E. coli* and *Klebsiella* isolates showing resistance to the other antibiotics were comparable. Comparison of

Figure 1: Comparison of pattern of antibiotic resistance in ESBL producing isolates obtained from in-patients and out-patients.

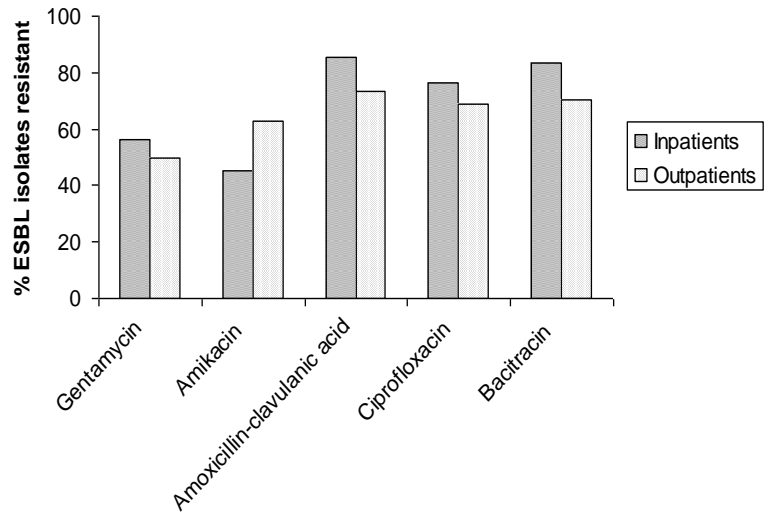


Table 1: Total number of ESBL and non-ESBL isolates isolated during the study period from in- and outpatients

Organism	In-patients		Out-patients	
	Total	ESBL (%)	Total	ESBL (%)
<i>E.coli</i>	690	109 (15.7)	4813	234 (4.8)
<i>K. pneumoniae</i>	225	31 (13.7)	955	30 (3.1)
<i>K. oxytoca</i>	12	3 (25)	55	2 (3.6)
Total	927	143 (15.4)	5823	266 (4.5)

antibiotic resistance profiles revealed that with the exception of amikacin, higher levels of resistance was demonstrated in ESBL-producing isolates obtained from in-patients (Figure 1)

Discussion

This is the first study from the Arabian Gulf region describing ESBL detection in *Enterobacteriaceae* isolated from all specimen types, from different patient populations with noscomial and community-acquired infections. Within the Arabian Gulf region, high ESBL prevalence of 31.7% in Kuwait and 41% in the United Arab Emirates [9,10] have been reported among inpatients. For Saudi Arabia, reported ESBL rates vary from 8.5-38.5% [4-8]. Thus in comparison to regional data, the finding of 6% ESBL producers in this study is on

the lower end of the spectrum. This finding is also similar to data reported from surveys in some countries in Europe and Asia [2,13,14].

Although a predominance of either *K. pneumoniae* or *E. coli* ESBL isolates has been identified in different geographical regions, *E. coli* has emerged as the major source of ESBLs in the community. In this study, the proportion of *E. coli* (83%) is higher compared to that found in other studies but similar predominance of *E. coli* has been reported in data from the United Arab Emirates [9,10]. Nosocomial infections caused by ESBL producing pathogens are associated with risk factors such as elderly age, prolonged hospitalization, previous antibiotic use, and presence of invasive devices [2,7]. The demographics in this study indicates that old age (> 60 years) remains a risk factor in our setting. As patients in critical care units are likely to have higher use of invasive devices such as urinary and vascular catheters, it is not surprising that up to 20% of ESBL isolates were from the intensive care unit. Urine was the most common specimen for the isolation of ESBL pathogens among in-patients and out-patients. Up to 74% of the total ESBL isolates obtained from out-patients were urinary isolates in comparison to the wider diversity of specimen types among in-patients.

However, the detection of ESBL producers among urinary *E. coli* isolates was significantly higher among in-patients. These findings have significant implications for empirical management of

Table 2: Distribution of ESBL isolates obtained from different body sites

Group	Body Site	ESBL positive isolates		Total (%)
		<i>E. coli</i>	<i>K. pneumoniae</i> & <i>K. oxytoca</i>	
In-patients				
	Urine	53	13	66 (46.1)
	Blood	23	9*	32 (22.4)
	Skin	8	3	11 (7.7)
	Surgical wound	10	2*	12 (8.4)
	Sputum	2	2	4 (2.8)
	Abscess	2	1	3 (2.1)
	Others	11	4*	15 (10.5)
	Total	109	34	143
Out-patients				
	Urine	182	16**	198 (74.4)
	Blood	10	5	15 (5.6)
	Skin	5	2	7 (2.6)
	Surgical wound	6	0	6 (2.3)
	Sputum	0	0	0 (0.0)
	Abscess	3	0	3 (1.1)
	Others	28	9	37 (14.0)
	Total	234	32	266

*Includes one *K. oxytoca* isolate ; ** Includes 2 *K. oxytoca* isolates

patients with urinary tract infection using third generation cephalosporins or fluoroquinolones, especially as we also found high levels of ciprofloxacin resistance in the ESBL isolates identified.

To our knowledge, this is the first report presenting data differentiating between nosocomial and community acquired ESBL infections (based on internationally defined criteria) in Saudi Arabia. About 60% of ESBL isolates were associated with nosocomial infections with a significantly higher proportion of *K. pneumoniae* isolated in nosocomial compared to community acquired infections. However, the proportion of *E. coli* in both settings was similar. These findings of occurrence of *E. coli*-producing ESBL isolates in the community is a cause for concern and is in keeping with the global trend of emergence of community-acquired infections caused by ESBL-producing strains, in particular those which harbour the CTX-M gene [15]. A recent report from Saudi Arabia showed 17.7% fecal carriage of ESBL isolates with the majority (over 80%) of these being *E. coli* [16]. This observation indicates that ESBL-producing *E. coli* are circulating within the community and are associated with infections emerging from these settings. Carbapenems are

widely regarded as the drugs of choice for treatment of infection caused by ESBL-producing organisms and remain useful in our setting as all isolates were sensitive to imipenem. As reduced imipenem susceptibility has been described in *E. coli* with CTX-M-type ESBL [17], we recommend molecular characterization of ESBL isolates circulating in our setting. In addition, continued antibiotic surveillance is needed to preserve the continued usefulness of these drugs. Our finding of high levels of resistance to non-beta lactam classes of antibiotics is in keeping with other reports and arises because these plasmid-mediated enzymes which are transferable between bacterial species also incorporate genetic material coding for resistance to other antibiotics [18]. Treatment failures have been reported with the use of beta-lactam/beta-lactamase inhibitor combinations for infections caused by ESBL-producing organisms [19]. The level of resistance reported here for amoxicillin-clavulanic is much higher than that reported in other studies [20,21] and also much higher than the 25% resistance to piperacillin-tazobactam among ESBL isolates described in United Arab Emirates [10], suggesting that this beta-lactam/ beta-lactamase inhibitor combination may not be useful in our setting.

In conclusion, the findings in this study document the emerging threat of ESBL pathogens in our setting with the occurrence of these strains as aetiological agents of infection in the hospital and community. While the findings shed light on *Klebsiella* species and *E. coli*, which are the predominant ESBL producers, we recommend further work on evaluating the ESBL types in these isolates as well as the prevalence of other ESBL-producing Gram negative bacteria which are emerging as pathogens of concern in the clinical setting. We advocate increased surveillance as well as comprehensive multicenter/ multinational studies to address this emerging problem of ESBL-associated infections.

Acknowledgement

This study was made possible by permission of the authorities of Saudi Aramco, Kingdom of Saudi Arabia. We acknowledge the kind assistance of the staff of the Preventive Medicine SVCS DIV/Epidemiology Unit at Saudi Aramco and Adnan Anani, Anthony Amalraj and Abdullah Wadei for their kind assistance in data collection. We thank Richard Sowers for reviewing the manuscript.

References

1. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S (1983) Transferable resistance to cefotaxime, ceftazidime, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 11: 315-317.
2. Bradford PA (2001) Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 14: 933-951.
3. Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 18: 657-686.
4. El-Khizzi NA, Bakheshwain SM. (2006) Prevalence of extended-spectrum beta-lactamases among *Enterobacteriaceae* isolated from blood culture in a tertiary care hospital. *Saudi Med J* 27: 37-40.
5. Kader AA, Angamuthu K (2005) 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. *Saudi Med J* 26: 956-959.
6. Kader AA, Kumar AK (2004) Prevalence of extended spectrum beta-lactamase among multidrug resistant Gram-negative isolates from a general hospital in Saudi Arabia. *Saudi Med J* 25: 570-574.
7. Panhotra BR, Saxena AK, Al-Ghamdi AM (2004) Extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* hospital acquired bacteremia. Risk factors and clinical outcome. *Saudi Med J* 25: 1871-1876.
8. Babay HA (2002) Detection of extended-spectrum beta-lactamases in members of the family enterobacteriaceae at a teaching hospital, Riyadh, Kingdom of Saudi Arabia. *Saudi Med J* 23:186-190.
9. Mokaddas EM, Abdulla AA, Shati S and Rotimi VO (2008). The technical aspects and clinical significance of detecting extended-spectrum beta-lactamase-producing enterobacteriaceae at a tertiary-care hospital in Kuwait. *J Chemother*;20:445-451..
10. Al-Zarouni M, Senok A, Rashid F, Al-Jesmi SM, Panigrahi D (2008) Prevalence and antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in the United Arab Emirates. *Med Princ Pract* 17: 32-36.
11. Centers for Disease Control National Nosocomial Study Site Definition Manual (1999) Center for Disease Control Atlanta GA .
12. Performance standards for antimicrobial susceptibility testing; 15th informational supplement (M100-S15) (2005) National Committee for Clinical Laboratory Standards, Wayne, Pa, USA, National Committee for Clinical Laboratory Standards.
13. Lewis MT, Yamaguchi K, Biedenbach DJ, Jones RN (1999) *In vitro* evaluation of cefepime and other broad-spectrum beta-lactams in 22 medical centers in Japan: a phase II trial comparing two annual organism samples. The Japan Antimicrobial Resistance Study Group. *Diagn Microbiol Infect Dis*; 35: 307-315.
14. Ishikawa K, Hayakawa S, Miyakawa S, Kusaka M, Shiroki R, Hoshinaga K (2005) Survey of the susceptibility of urinary isolates to antibacterial agents in 2003. *J Infect Chemother* 11: 44-47
15. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N (2007) CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* 59:165-174.
16. Kader AA, Kumar A, Kamath KA. (2007) Fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in patients and asymptomatic healthy individuals. *Infect Control Hosp Epidemiol* 28:1114-1116.
17. Lartigue M-F, Poirel L, Poyart C, Réglie-Poupet H, Nordmann P (2007) Ertapenem Resistance of *Escherichia coli*. *Emerg Infect Dis* 13:2.
18. Paterson DL (2006). Resistance in gram-negative bacteria:*Enterobacteriaceae*. *Am J Infect Control* 34: S20-28.
19. Peterson LR (2008) Antibiotic policy and prescribing strategies for therapy of extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: the role of piperacillin-tazobactam. *Clin Microbiol Infect Suppl* 1: 181-184
20. Mehrgan H, Rahbar M (2008) Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* in a tertiary care hospital in Tehran, Iran. *Int J Antimicrob Agents* 31:147-151.
21. Sorlozano A, Gutierrez J, Romero JM, de Dios LJ, Damas M, Piedrola G (2007) Activity in vitro of twelve antibiotics against clinical isolates of extended-spectrum beta-lactamase producing *Escherichia coli*. *J Basic Microbiol* 47:413-416.

Corresponding author

Dr. Abiola Senok
 Department of Clinical Sciences
 College of Medicine, University of Sharjah
 PO Box 27272, Sharjah, United Arab Emirates
 Tel: +971 (6) 5057220 Fax: +971 (6) 5585879
 Email: asenok@sharjah.ac.ae

Conflict of interest: No conflict of interest is declared.