Prevalence of extended–spectrum beta–lactamases among *Enterobacteriaceae* isolated from blood culture in a tertiary care hospital

Noura A. El-Khizzi, *MD, FRCPath (Micro),* ***Saadia M. Bakheshwain,*** *MD, KSUFPath (Micro).*

ABSTRACT

Objectives: To determine the prevalence of extended spectrum ß-lactamase among *Enterobacteriaceae* isolated from blood culture in a tertiary care hospital.

Method: We carried out this study at the Armed Forces Hospital, Riyadh, Kingdom of Saudi Arabia during the period between January 2003 – December 2004. We tested a total of 601 isolates of the family *Enterobacteriaceae* from blood culture for the prevalence of extended spectrum ß-lactamase (ESBL) production by the standardized disc diffusion method and confirmed by the ESBL E test strips.

Results: Ninety-five (15.8%) of the isolates were ESBL producers. Among these, 48.4% were *Klebsiella pneumoniae (K. pneumoniae)* followed by15.8% of both *Escherichia coli (E. coli)* and *Enterobacter cloacae (Ent. cloacae)*. Other isolates produced ESBL in low numbers.

Conclusion: *Klebsiella pneumoniae* produced ESBL in significant numbers. Extended spectrum ß-lactamase gram-negative bacilli present significant diagnostic and therapeutic challenges to the management of infections due to these organisms. Microbiology laboratories should start reporting ESBL producing *Enterobacteriaceae* organism due to their importance in respect to antibiotic therapy and infection control aspects.

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Beta-lactam antibiotics are among the safest and most frequently prescribed antimicrobial agents worldwide. The emergence of resistance to these agents in the past 2 decades has resulted in a major clinical crisis.[1,2] Gram-negative bacteria resistant to agents such as extended-spectrum cephalosporins, monobactam, ß-lactam, ß-lactamase inhibitors combinations and carbapenems have emerged through the production of variety of ß-lactamases, alteration in the penicillin binding proteins, outer membrane permeability, and combination of multiple mechanisms of resistance. This increase has paralleled the introduction, administration and over use of ß-lactam.[3]

Extended-spectrum ß-lactamases (ESBLs) are primarily plasmid-mediated enzymes frequently derived from TEM or SHV-related enzyme. Both TEM-1 and SHV-1 are parent enzymes that confer resistance to ampicillin.[4]

The ESBL-producing isolates are resistant not only to amino penicillin, ureidopenicillins and narrow-spectrum cephalosporins, but all extended-spectrum cephalosporin and aztreonam.[5] Cephamycins and carbapenem retain activity against these isolates.

From the Department of Pathology, Armed Forces Hospital, Riyadh, *Kingdom of Saudi Arabia.*

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Address correspondence and reprint request to: Dr. Noura A. El-Khizzi, Consultant Microbiologist, (B-93), Armed Forces Hospital, PO Box 7897, Riyadh 11159, *Kingdom of Saudi Arabia.* Tel. +966 (1) 4791000 Ext. 4456. Fax. +966 (1) 4783033. E-mail: naael@hotmail.com

Over 120 different ESBL types have been identified, with each type conferring a slight different susceptibility profile, complicating selection of therapy.[6]

Emergence of ESBL-producing isolates has important clinical and therapeutic implication. First, in most bacterial isolates, resistance determinants for ESBL production are carried on plasmids that can be easily spread from organism to organism.[5] Second, the spread of resistance toward extended-spectrum cephalosporins further limits the utility of the ß-lactam class and may lead to increased prescription of more broad-spectrum and expensive drugs such as imipenem. In addition, this isolates may escape detection with routine susceptibility testing performed by a clinical microbiology laboratory, which can result in adverse therapeutic outcomes.[7,8] More important, antibiotic selection for treatment of serious infection due to ESBL-producing *Enterobacteriaceae* is a clinical challenge due to the complex nature of in-vitro susceptibility testing and vivo correlation. Perhaps the biggest challenge lies in overcoming widespread unawareness among clinicians regarding these resistant organisms due to underreporting by microbiology laboratories and lack of an obvious marker to indicate production of an ESBL.[9]

Extended-spectrum ß-lactamase (ESBL) producing organisms pose unique challenges to clinical microbiologists, clinicians, infection control professionals and antibacterial-discovery scientists. These ESBL-producing resistant pathogens include *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Enterobacter cloacae (Ent. cloacae), Serratia marcescens (S. marcescens), Citrobacter freundii (C. freundii), Pseudomonas aeruginosa (P. aeruginosa) and Acinetobacter baumannii (A. baumannii).*

The objective of this study was to determine the prevalence of ESBL-producing *Enterobacteriaceae* isolated from blood cultures in a tertiary care hospital.

Methods. This study was carried out at the Clinical Microbiology Department of the Armed Forces Hospital, Riyadh, Kingdom of Saudi Arabia, a 1200 bed, tertiary care facility. A total of 601 non-duplicate *Enterobacteriaceae* isolated from blood culture during the period of the study from January 2003 to December 2004 were reviewed. The isolates were identified by standard microbiological techniques, API 20E (bioMerieux, Marcy I'Etoile, France). Sensitivity testing was carried out using standardized disk diffusion method[10] for ampicillin (10 μg), amoxicillin-clavulanic acid (30 μg), sulfamethoxazole-trimethoprim (2.5-50 μg) cefuroxime (30 μg), ceftriaxone (30 μg) ceftazidime (30 μg) aztreonam (30 μg), ciprofloxacin (5 μg), tazobactam/piperacillin (75/10 μg), cefepime (30 μg), gentamicin (10 μg), netilmicin (30 μg), amikacin (30 μg), imipenem (10 μg), meropenem (10 μg) colistin (25 μg) (Oxoid, Basingstoke, United Kingdom) *P. aeruginosa* ATCC (27853), and *E. coli* ATCC (35218) were used as control strains.

Isolates with intermediate or resistant susceptibility for extended-spectrum cephalosporin and aztreonam were retested using ESBL E-test strip for confirmation of ESBL production (AB Biodisk, Piscataway, New Jersey). E-test strips with gradient concentration of ceftazidime (CAZ) or cefotaxime (CTX) at one end and CTX or CAZ with clavulanic acid (CA) at the other end was performed in accordance with the manufacturer recommendation. The ESBL production was confirmed by a ratio of CAZ or CTX minimum inhibitory concentration (MIC) to CAZ or CTX with CA of >8. The ESBL production was also identified by the presence of a phantom zone, or a deformity of strip, a non-determinable (ND) was declared when MICs were greater than the range of strip.

Table 1 - Frequency of *Enterobacteriaceae* producing ESBL among the isolates (N=601).

Organism	No. of isolates	Organism (%)	No. of ESBL Producers	ESBL Producers (%)
Klebsiella pneumoniae	206	(34.3)	46	(48.4)
Escherichia coli	195	(32.4)	15	(15.8)
Enterobacter cloacae	80	(13.3)	15	(15.8)
Enterobacter aerogenes	18	(3)	6	(6.3)
Citrobacter freundii	7	(1.2)	5	(5.3)
Serratia marcescens	25	(4.1)	4	(4.2)
Proteus staurti	6	(1)	2	(2)
Klebsiella oxytoca	21	(3.5)	1	(1.1)
Proteus mirabilis	18	(3)	1	(1.1)
Enterobacter sakazaki	6	(1)	0	-
Serratia liquifans	4	(0.7)	0	-
Salmonella species	15	(2.5)	0	-
Total	**601**	**(100)**	**95**	**(100)**

ESBL - extended spectrum ß-lactamase

Results. Of the 601 isolates of the members of the family *Enterobacteriaceae, K. pneumoniae* was the most frequent organism isolated 206 (34.3%) followed by *E. coli* 195 (32.4%), *Ent. cloacae* 80 (13.3%) and *S. marcescens* 25 (4.1%) **Table 1**. Extended spectrum ß-lactamase production was confirmed by ESBL E-test strip in 95 isolates making a prevalence of 15.8%. *Klebsiella pneumoniae* was the most ESBL producer 48.4%, followed by both *E. coli* and *Ent. cloacae* each 15.8%, *Enterobacter aerogenes (Ent. aerogenes)* produced ESBL at lower rate at 6.3%, *C. freundii* at 5.3% and *S. marcescens* at 4.2%.

Klebsiella oxytoca, Proteus mirabilis (P. mirabilis) and Proteus stuarti (P. stuarti) produced minimal ESBL (**Table 1).**

Enterobacter sakazaki, S. liquifans, and Salmonella species did not produce any detectable ESBL. All ESBL producing isolates were sensitive to carbapenems.

Discussion. Resistance to ß-lactam antimicrobial agents, especially extended-spectrum cephalosporins and other antimicrobial, among clinical isolates of gram-negative bacteria is increasing worldwide.[11,12] Reports of clinical failure and nosocomial infections due to ESBL are emerging.[13-15] Risk factors for infection with ESBL producing organisms included prolonged hospitalization, the use of various invasive diagnostic producers and prior antibiotic treatment included ß-lactam agents.[16]

The emergence of ESBL has created not only a diagnostic problem but also poses a potential therapeutic challenge for the use of ß-lactam in serious infections by the *Enterobacteriaceae*. It has also implications for nosocomial infections in intensive care unit and other special care units where the use of extended-spectrum ß-lactams is high. According to a survey by the National Committee for Clinical Laboratory Standards the prevalence of ESBL is probably underestimated.[17] The prevalence of ESBL-producing isolates among *Enterobacteriaceae* range from a national average of 3% in the United States[18] to a much higher numbers in Europe where the prevalence of ESBL production among isolates varies greatly from country to country. In the Netherlands, a survey of 11 hospital laboratories showed that <1% of *E. coli* and *K. pneumoniae* strains possessed an ESBL.[19] However, in France as many as 11.4% of *K. pneumoniae* and 47.7% of *Ent. aerogenes* were found to be ESBL producers.[20]

The reports on ESBL production by *Enterobacteriaceae* from Saudi Arabia are few. Babay[21] reported ESBL production in 36% of their isolates, Bilal and Gedebou[22] detected ESBL in 27.5% of *K. pneumoniae* in Abha, Kader and Kumar[23] reported ESBL in 4.8% of their gram negative isolates. The prevalence of ESBL among our isolates was 15.8%. This is lower than some reported results from Saudi Arabia.[21,22] Higher rates were reported from India 68%[24] and Pakistan 45%.[25]

We can explain the variation in the prevalence of production of ESBL among our isolates by the fact that our isolates were mainly from blood culture while in the other studies it was from different sites, wounds, urine, tracheal aspirates, and others. The majority of the ESBL producers among our isolates were *K. pneumoniae* (48.4%) and this is similar to other findings from Riyadh (42%),[21] United States[26] and Argentina (48%)[27] where *K. pneumoniae* was the most common ESBL producers.

Escherichia coli and *Ent. cloacae* produced ESBL at similar rate 15.8% and although these are reported to be high ESBL producers by other studies,[21,28] it did not seem to be so in our study. Other *Enterobacteriaceae* produced ESBL in low numbers.

In conclusion, data regarding the prevalence of ESBL strains in Saudi Arabia is limited.[23,29] We can increase the level of information regarding the prevalence of ESBL by conducting studies in hospitals in Saudi Arabia, and providing data for clinicians and also for comparing the prevalence of ESBL-producing of gram-negative at both local and national levels.

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