Detection of extended-spectrum β-lactamases in members of the family enterobacteriaceae at a teaching hospital, Riyadh, Kingdom of Saudi Arabia

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ABSTRACT

Objective: To determine the prevalence of extended spectrum β-lactamase at King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia and to compare the ability of the disc diffusion, double disc potentiation methods and the extended-spectrum β-lactamase E test to detect exended-spectrum β-lactamase among enterobacteriaceae.

Methods: This study was undertaken during the time period period January 1 through to September 30 1999, at King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia. A total of 187 multiresistant isolates of enterobacteriacae from different clinical specimens were tested for the extended-spectrum β-lactamases. The performance of disc diffusion, double disc potentiation methods using cefotaxime, ceftazidime, ceftriaxone and aztreonamand the extended-spectrum β-lactamase E based on the reduction of the minimum inhibitory concentration of ceftazidime in the presence of clavulanic acid were compared for the detection of extended-spectrum β-lactamase production.

Results: Thirty six percent of our isolates produced extended-spectrum β-lactamases. Among these 42% were *Klebsiella pneumoniae* and 20% were *Escherichia coli*. Other species of Enterobacteriaceae produced extended-spectrum β-lactamase in low numbers. Disc diffusion method was not suitable for detecting extended-spectrum β-lactamases among the isolates. Double disc potentiation performed comparably well with the extended-spectrum β-lactamases E test.

Conclusion: Extended-spectrum β-lactamases occurs in *Klebsiella pneumoniae* and *Escherichia coli* at a significant number. The use of double disc potentiation method for screening is practical and the extended-spectrum β-lactamase E test with ceftazidime is a useful confirmatory test for extended-spectrum β-lactamase production.

Keywords: Extended-spectrum β-lactamase, ceftazidime, double disc potentiation E test enterobacteriaceae test.

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Plasmid-borne β–lactamase conferring resistances to the extended-spectrum cephalosporins were first recognized in Germany in 1983.[1] Extended-spectrum β-lactamase (ESBL) enzymes hydrolyse the extended-spectrum cephalosporins like cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO) and the monobactam aztreonam (ATM). Extended-spectrum β-lactamase are most commonly found in *Klebsiella pneumoniae (K.pneumoniae)* and *Escherichia coli (E.coli)* but they have also been detected among other species of the family Enterobacteriaceae.[2] Extended-spectrum β-lactamase that are plasmid mediated are distinct from chromosomal types with overlaps. It can be predicted

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by susceptibility to β-lactamase inhibitors such as clavulanic acid or sulbactam and cephamycin such as cefoxitin (Fox).[2-5] Plasmid mediated AmpC also occurs and is called ESBL but, is very rarely reported.[2-5] Extended-spectrum β-lactamase are easily overlooked due to the low-level resistance to extended-spectrum cephalosporins and ATM.[2,4,6] This low-level resistance probably reflects lesser enzyme quantity.[5] Many specific detection methods have been developed to detect ESBL including double disc potentiation (DDP), the 3 dimensional methods (3DM), vitek rapid automated procedure and E test ESBL strip.[7-10] In 1999 the National Committee for Clinical Laboratory Standards (NCCLS) described screening and modified breakpoints for disc diffusion (DD) and confirmatory tests for detecting ESBL producing *K.pneumoniae, K.oxytoca* and *E.coli*.[11]

Reports of outbreaks of nosocomial infections resistant to broad spectrum β–lactam antibiotics and treatment failure are increasing.[12-14] In the Kingdom of Saudi Arabia Bilal et al[15] detected ESBL in 27.5% of clinical strains of *K.pneumoniae* using DDP method in a maternity hospital in Abha in 1999. El-Karsh et al[16] in 1995 detected only one ESBL producing *K.pneumoniae* among 106 gram-negative bacilli from the intensive care unit in our hospital by isoelectric point. This study was undertaken to determine the prevalence of ESBL, and compare the ability of DD method currently used in our laboratory with the DDP method and the ESBL E test strip to detect ESBLs among enterobacteriaceae isolated in our hospital. There has been no previous report of ESBL detection among enterobacteriaceae in the Kingdom of Saudi Arabia utilizing these 3 methods.

Methods. The study was undertaken in King Khalid University Hospital, a 600 bed tertiary hospital in Riyadh, Kingdom of Saudi Arabia, from January 1st 1999 through to September 30 1999. A total of 187 isolates from in patients were examined. The isolates were all resistant to the 3rd generation cephalosporins and ATM. Some of them were also resistant to aminoglycosides, ciprofloxacin and imipenem. Clinical data was obtained from the charts of all patients. These include wounds, urinary tract infections, septicemia and chest infections. The patients were considered infected or colonized based on clinical data. The organisms were routinely tested for susceptibility by DD using the Stoke's comparative disc diffusion method.[17] Standard reference strain of *E.coli* (ATCC (25922) was used as a control. The antibiotics tested were: Ampicillin (10μg), augmentin (30μg), cephradine (30μg), cefuroxime (30μg), FOX (30μg), CRO (30μg) CTX (30μg), CAZ (30μg), ATM (30μg) ciprofloxacin (5μg), sulfamethoxazole-trimethoprim (2.5μg-50μg), gentamicin (10μg), amikacin (30μg) and imipenem (10μg) (Mast diagnostics, Bootle, Merseyside L20 (IEA, United Kingdom). Isolates resistant (and intermediate resistant) to the extended-spectrum β-lactam agents were further tested for ESBL production by DDP method, and confirmed by ESBL E test strip test.

Double disk potentiation test. Mueller-Hinton agar plates (MH II agar BBL. (Becton Dickinson and Company, Cockeysville, Maryland, United States of America) was inoculated with the test organisms grown overnight on blood agar plates as for the DD method. Cefotaxime (30μg) CAZ (30μg), CRO

Table 1 - Comparison of disc diffusion, double disc potentiation and extended spectrum β-lactamase E test for detecting extended spectrum β-lactamase producing enterobactericae.

Species (N of isolates)	N of resistant isolates detected by DD method				N of ESBL detected by DDP methods				N of ESBL detected by ESBL E test
	CTX	CRO	CAZ	ATM	CTX	CRO	CAZ	ATM	TZ/TZL >8
K.pneumoniae (64)	63	64	64	64	41	40	42	42	42
K.oxytoca (1)	1	1	1	1	0	0	0	0	0
E.coli (69)	69	69	69	69	24	23	23	23	20
C.freundii (12)	12	11	12	12	2	1	1	1	1
C.diversus (4)	4	4	4	4	0	0	0	0	0
Ent.cloacae (21)	21	21	21	21	0	0	0	0	0
Ent.agglumerance (5)	5	5	5	5	0	1	1	1	1
Ent. aerogenes (3)	3	3	2	3	1	1	0	1	1
Ent.sakazaki (4)	3	4	4	3	1	0	1	1	0
S.marcescens (1)	1	1	1	1	1	1	1	1	1
M.morganii (2)	2	2	2	2	0	0	0	0	0
P.vulgaris (1)	1	1	1	1	1	1	1	1	1
Total (187)	**184 (98.9)**	**186 (99.4)**	**186 (99.4)**	**185 (98.9)**	**70 (37.4)**	**67 (35.8)**	**70 (37.4)**	**71 (37.9)**	**67 (35.8)**

N=number, DD=disc diffusion,CTX=cetotaxime, CRO=ceftriaxone, CAZ=ceftazidime, ATM=aztreonam, ESBL=extended spectrum β-Lactamase, DDP=double disc potentiation, TZ=cetazidime, TZL=cetazidime clavulanic acid, K=Klebsiella, E=Escherichia, C=Citrobacter, Ent=Enterobacater, S=Serratia, M=Morganella, P=proteus

(30µg), and ATM (30µg) disks were placed 30mm (center to center) from amoxicillin – clavulanate (20 µg and 10µg) disk as recommended by Jarlier et al.[18] After overnight incubation in air at 35ºC, presumptive evidence for the presence of an ESBL was indicated by enhancement of the zone of inhibition of the ESBL antibiotic nearer to the amoxicillin-clavulanate disc. This was recorded as DDP test positive.

Extended-spectrum β-lactamase E test strip. All positive DDP test isolates were confirmed for the production of ESBLs by ESBL E test strip (obtained from AB Biodisk Solna, Sweden). The test was performed on the Mueller-Hinton agar plate according to the manufacturers instructions. The ratio of CAZ minimum inhibitory concentration (MIC) with clavulanic acid and CAZ alone (TZ/TZL) of > 8 indicate the presence of ESBL activity.

Results. Of the 187 multiresistant isolates of members of the family enterobacteriacae tested by DD method, the majority were *E.coli* 69 (37%) and *K.pneumoniae* 64 (34%), **(Table 1).** The only *K. oxytoca* isolated did not produce any detectable ESBL by both methods. Resistance to extended spectrum β–lactams was detected by DD method in 99.4% of isolates to CRO, CAZ and in 98.9% to ATM and CTX, of which an average of 36% produced ESBL by DDP and ESBL E tests, **Table 1.** *K.oxytoca*, Citrobacter diversus *(C.diversus),* Enterobacter cloacae *(Ent.cloacae)* and Morganella morganii *(M.morganii)* did not produce any detectable ESBL by both methods. As shown in **Table 2.** Sixty-five point six percent of *K. pneumoniae* and 29% of *E.coli* isolates produced ESBL. Only 0.5% each of the other members of the Enterobacteriaceae produced ESBL. Sixty percent (113) of isolates were fox susceptible. Over 95% (179) of the isolates were susceptible to imipenem.

Extended-spectrum β-lactamases producing organisms were recovered from various specimens **Table 3.** The majority were from urine specimens of patients with urinary tract infections. Twenty-one point three percent were from wound infections including diabetic wounds. Of the isolates from bacteremic patients, 5 were *K.pneumoniae* from blood of patients with chronic renal failure, 3 were *E. coli* from 2 post cholecystectomy patients and one with colonic cancer. Of the ESBL producing organisms 6.4% were from chest infections in various intensive care units.

Discussion. Our ESBL producing isolates were from 6 different members of the Enterobacteriaceae, an observation consistent with other reports.[8,9] Although the DD method did not produce any discernible pattern of reduced susceptibility for detecting ESBL production in the 187 isolates tested, 67 (36%) were found to be ESBL producers by using the additional tests of DDP and ESBL E test. Moland et al[19] concluded in their study that cephamycin-susceptible strains of *E.coli* and *K.pneumoniae* or *K. oxytoca* are highly likely to produce ESBL. Vercauteren et al[7] reported that 48% of ESBL producing isolates had reduced susceptibility to CAZ by DD method. In contrast, Jacoby and Han[4] reported that DD method underestimates the presence of ESBL producing strains. According to Brown et al,[20] the inability of DD method to detect ESBL producing isolates could be due to another mechanism mediating this resistance. Disc diffusion is able to detect ESBLs when the genes encoding for them are clustered with those for aminoglycoside, sulfonamides, tetracycline and other antibiotics therefore the finding of resistance to these agents in

Table 2 - Isolates with extended spectrum β-lactamase.

Isolates	N (%) of isolates tested	N (%) of isolates with ESBL
K.pneumoniae	64 (34)	42 (65.6)
E.coli	69 (37)	20 (29)
C.freundii	12 (6)	1 (8.3)
S.marcescens	1 (0.5)	1 (100)
Ent.agglumerance	5 (3)	1 (20)
E.aerogenes	3 (2)	1 (33.3)

N=number, ESBL=extended spectrum β-lactamase, K=klebsiella, E=escherichia, C=Citrobacter, S=serratia, Ent=enterobacter

Table 3 - Sites of isolation of ESBL producing organisms.

Sites of isolation	N (%) of patients
Urine/Catheter Urine	95 (51)
Pus/abdominal swabs	43 (22.9)
Sputum/Tracheal Aspirate	24 (12.8)
Blood	10 (5.34)
Others	15 (8)

N=number, ESBL=extended-spectrum β-lactamase

E.coli or *K.pneumoniae* should alert the laboratory to the need for further testing.[21]

Although the DDP method is reliable for the detection of most ESBLs in *E.coli* and *K. pneumoniae,* it is more subjective than the E test and false negatives may occur due to strains that produce enzymes that are not inhibited by clavulanic acid not being detected.[20] In addition ESBLs that are not active against CAZ will not be detected unless additional agents are tested as in DDP method.[5] Cormican et al[6] reported that DDP method has a 79% sensitivity compared to the 95% of the 3DM for detecting ESBLs and that the E test had a 100% sensitivity compared to the DDP method. In our study, DDP method performed comparably with E test. It detected an average of 67 (36%) of ESBLs by CAZ, CTX, CRO and ATM disks. The sensitivity of DDP strongly depends on the distance between the CAZ disk and the amoxicillin-clavulanic acid during the test.[7] This has been found to be between 20-30mm for all TEM-related ESBL enzymes except TEM-12.[7]

Various extended-spectrum cephalosporins have been tested including CRO, CTX, cefepime and cefpodoxime using DDP or 3DM.[4,7,8,19,21] Most investigators have suggested the use of CAZ as the best indicator for ESBL or AMPC β–lactamase.[6,19,21] Jacoby and Han used CAZ-5 mg disk and reported that a zone of inhibition of 20mm or less detected ESBL production in *E.coli* and *K.pneumoniae*.[4] Coudron et al[8] detected 6 of 7 ESBL producing isolates *(E.coli or K.pneumoniae)* with a zone of inhibition of > 20mm by using CAZ – 5mg disk. Although it is expensive, the ESBL E test has been reported by many investigators to have high sensitivity and to be convenient.[6,7] In our study, the results of the DDP method were comparable to the ESBL E test although augmentation was more easily seen by the E test. In contrast, Vercauteren et al[7] reported that out of the 33 ESBL producing *E.coli* and *K.pneumoniae,* 81.2% were detected by the E test when an MIC equal to or greater than 8μg/ml is used as an indicators of ESBL production among these isolates. Cormican et al[6] proposed that if the breakpoint for ESBL production is reduced to 5μg/ml instead of 8μg/ml, more strains will be detected and the sensitivity increased to 87.5%. Extended-spectrum β-lactamases E test is very effective for the detection of TEM related ESBL enzymes.[7] M'Zali et al[22] compared MAST double disc as well as the double DD and the E tests. He found that the MAST double disc is 86% sensitive for detecting ESBL using disc containing CAZ but 65.5% when using CTX and the result increased to 93% if both discs were used.[22] He reported that using a single disc can result in missing ESBL producing isolates and that both the E test and the MAST double disc methods identified 93% of ESBL producers.[22]

The emergence of ESBL has created not only a diagnostic problem but also poses a potential therapeutic challenge for the use of β–lactams 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 β–lactams is high. Reports of clinical failure and nosocomial infections due to ESBL are emerging.[12-14] In our study infections with ESBL producing isolates were seen in seriously ill patients and necessitated a change of antibiotics to the carbepenems such as imipenem. Risk factors of infection with ESBL producing organisms included prolonged hospitalization, young age, the use of various invasive/diagnostic procedures and prior antibiotic treatment included β–lactam agents.[12] Treatment failure with the ESBL is likely in a patient who has a high concentration on ESBL producing organism (>10^7 cfu/ml) in an infection site.[14]

The true extent of the problem of ESBL is under recognized. The reason for this is poor detection, and many laboratories may be unaware of the NCCLS guidelines on modified susceptibility testing for ESBL producing strains.[2] In this study we were able to detect as many as 36% ESBL producing strains of *K.pneumoniae* and *E.coli* in our hospital. The identity of ESBL enzymes produced by enterobacter sp, citrobacter sp and *Morganella morganii* (M.morganii) was not performed in this study since these organisms produce AmpC β–lactamases which are induced by clavulanate but not inhibited by this inhibitor. Therefore, DDP and E tests may not predict ESBL production among these organisms. In addition these organisms did not produce significantly detectable ESBL in our study. There is controversy in the cost-effectiveness of detection of ESBL producing strains. Emery and Weymouth[9] reported that the detection on routine bases is not clinically or cost effective when the prevalence of ESBL is low. Moland et al[19] also disagree on routine testing for ESBL as differentiation between ESBL and AmpC producing strains is not critical and therapeutic options for patients infected with such strains are limited. The cost comparison of the DDP and ESBL was very high in this study. Extended-spectrum β-lactamases test costed 320 dollars compared to 30 dollars for the DDP. Each test required one agar plate for each organisms. Furthermore, the results of the 2 tests were similar. Therefore the use of the DDP is economical and cost-effective compared to the ESBL test coasted.

In conclusion this data indicates that ESBL occurs in *K.pneumoniae* and *E.coli* at a significant level in our hospital with potentially serious clinical implications for antimicrobial chemotherapy. The DDP performed comparably well with the E test. We recommend that microbiology laboratories should be

aware of the problem of ESBL and it may be clinically cost-effective to detect its production in selected cases using DDP method. Clinicians should consider the probability of treatment failures with extended β–lactams and change treatment to a more appropriate antibiotic such as the carbepenems.

References

1. Knothe H, Shah P, Kremery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of K. pneumoniae and S. marcescens. *Infection* 1983; 11: 315-317.
2. Tenover FC, Mohammed MJ, Gorton TS, Dembek ZF. Detection and reporting of organisms producing Extended-Spectrum β–Lactamases: survey of Laboratories in Connecticut. *J Clin Microbiol* 1999; 37: 4065-4070.
3. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β–lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995; 39: 1211-1233.
4. Jacoby GA, Han P. Detection of Extended-Spectrum β–Lactamases in clinical isolates of K. pneumoniae and E. coli. *J Clin Microbiol* 1996; 34: 908-911.
5. Livermore DM. β-lactamases in Laboratory and clinical resistance. *Clin Microbiol Rev* 1995; 8: 557-584.
6. Cormican MG, Marshall SA, Jones RN. Detection of Extended-Spectrum β–Lactamase (ESBL) producing strains by the E test ESBL screen. *J Clin Microbiol* 1996; 34: 1880-1884.
7. Vercauteren E, Descheemaeker P, Ieven M, Sanders CC, Goossens H. Comparison of screening methods for detection of extended-spectrum β–lactamases and their prevalence among blood isolates of E. coli and Klebsiella spp in a Belgian teaching hospital. *J Clin Microbiol* 1997; 35: 2191-2197.
8. Coudron PE, Moland ES, Sanders CC. Occurrence and detection of extended-spectrum β–lactamases in members of the family Enterobacteriaceae at a Veterans Medical Center: Seek and you may find. *J Clin Microbiol* 1997; 35: 2593-2597.
9. Emery C, Weymouth LA. Detection and Clinical significance of extended-spectrum β–Lactamases in tertiary care medical center. *J Clin Microbiol* 1997; 35: 2061-2067.
10. Sanders CC, Washington JA, Barry AL, Shubert C. Assessment of the Vitek ESBL test. In: Program and abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. [Abstract D44] Washington DC (USA): Amercian Society for Microbiology; 1994. p. 123.
11. National Committee for Clinical Laboratory Standards 1999. Performance Standards for antimicrobial susceptibility testing; ninth informational supplement. M100-S9. National Committee for Clinical Laboratory Standards. Wayne (PA).
12. Pagani L, Perilli M, Migliavacca R, Lazzaro F, Amicosante G. Extended-Spectrum TEM and SHV-type β–Lactamase-producing K. pneumoniae strains causing outbreaks in intensive care units in Italy. *Eur J Clin Microbiol Infect Dis* 2000; 19: 765-772.
13. Meyer KS, Urban C, Eagan JA, Berger BJ. Nosocomial outbreak of Klebsiella infection resistant to late-generation cephalosporins. *Ann Intern Med* 1993; 119: 353-358.
14. Karas JA, Pillay DG, MucKart D, Sturm AW. Treatment failure due to extended spectrum β–lactamase. *J Antimicrob Chemother* 1996; 37: 203-204.
15. Bilal NE, Gedebou M. Clinical and community strains of K. pneumoniae: multiple and increasing rates of antibiotic resistance in Abha, Saudi Arabia. *Br J Biomed Sci* 2000; 57: 185-191.
16. El-Karsh T, Tawfik AF, Al-Shammry F, Al-Salah S, Kambal AM, Shibl AM. Antimicrobial resistance and prevalence of extended-spectrum β–lactamase among clinical isolates of Gram- negative bacteria in Riyadh. *J Chemother* 1995; 7: 509-514.
17. Stokes EJ, Ridgway GL, Wren MWD. Clinical Microbiology, 7th ed. London, (UK): Edward Arnold; 1993. p. 234-280.
18. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum β–lactamases conferring transferable resistance to newer β–lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10: 867-878.
19. Moland ES, Sanders CC, Thomson KS. Can results obtained with commercially available Micro Scan Micro dilution Panels serve as an indicator of β–lactamase production among E. coli and Klebsiella isolates with hidden resistance to expanded-spectrum cephalosporins and aztreonam? *J Clin Microbiol* 1998; 36: 2575-2579.
20. Brown DF, Andrew J, King A, MacGowan AP. Detection of extended-spectrum β–lactamases with E test and double-disc potentiation methods. *J Antimicrob Chemother* 2000; 46: 327-328.
21. Katasanis GP, Spargo J, Ferraro MJ, Sutton L, Jacoby GA. Detection of K. pneumoniae and E. coli strains producing extended-spectrum β–lactamases. *J Clin Microbiol* 1994; 32: 691-696.
22. M' Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of extended-spectrum β–lactamases in members of the family Enterobacteriaceae: comparison of the MAST DD test, the double disc and the E test ESBL. *J Antimicrob Chemother* 2000; 45: 881-885.