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Prevalence of Extended-Spectrum Beta-Lactamases in Enterobacteriaceae, *Pseudomonas* and *Stenotrophomonas* as Determined by the VITEK 2 and E Test Systems in a Kuwait Teaching Hospital

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Key Words
Prevalence · Extended-spectrum β-lactamases · VITEK 2 · E test

Abstract
Objective: To determine the prevalence of extended-spectrum β-lactamase (ESBL)-producing members of the Enterobacteriaceae using VITEK 2 and E test systems. ***Materials and Methods:*** A total of 3,592 consecutive gram-negative isolates (single isolate per patient) of the family of Enterobacteriaceae and *Pseudomonas* adjudged to be clinically relevant to the patient's infection were studied for ESBL production over a period of 1 year at Mubarak Al-Kabeer Hospital, Kuwait. Two methods were used: the automated VITEK 2 system and E test ESBL, a manually manipulated plastic strip containing various gradients of β-lactam antibiotics. These tests and interpretative criteria for the results were performed according to the manufacturer's instructions. ***Results:*** Of the 3,592 bacterial isolates, 264 (7.5%) and 185 (5.2%) were positive for ESBL production by the VITEK 2 and E test, respectively. All the ESBL-producing *Pseudomonas aeruginosa* identified by VITEK 2 gave indeterminate results by E test. Prevalent ESBL producers, identified by the VITEK 2 versus E test, respectively, were: *Citrobacter* spp. (15 vs. 3.2%), *K. pneumoniae* (12.2 vs. 11.4%), *Enterobacter* spp. (12 vs. 3%), *E. coli* (6.5 vs. 5.6%), *P. aeruginosa* (6.5 vs. 0%) and *Morganella* spp. (2 vs. 1%). The most common infection associated with ESBL-producing pathogens was urinary tract infection (68.2%), followed by wound infection (14.4%) and bloodstream infection (6.1%). ***Conclusion:*** The result of this study showed a relatively high prevalence of clinically significant ESBL producers among the Enterobacteriaceae and *Pseudomonas* spp. at our teaching hospital. The VITEK 2 identified a higher prevalence of ESBL strains than the E test.

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Introduction

A multitude of evidence in the literature has shown a worldwide increase in bacterial resistance to ß-lactam antibiotics. Antibiotic resistance is complex and dynamic. This is because new factors, apart from the genetic and biochemical mechanisms already recognized, continue to be discovered. The spread of bacterial resistance to some important antibiotics commonly used in the hospital to treat infections, some life-threatening, is partly related to widespread dissemination of plasmid-mediated extended-spectrum ß-lactamases (ESBLs). ESBLs are enzymes that have the ability to hydrolyze oxyimino-aminothiazolyl cephalosporins, e.g. cefuroxime, ceftazidime, cefotaxime, ceftriaxone and cefipime as well as penicillin, monobactam and other cephalosporins except cephamycins [1]. They are often seen in *Klebsiella pneumoniae* and *Escherichia coli*, especially in nosocomial infections in the intensive care units (ICU) and other specialized units such as oncology, burn, neonatal wards, and in infections produced by indwelling devices, and are rarer in other members of the Enterobacteriaceae [2]. Several ESBLs have also been found in *Pseudomonas aeruginosa*, but many of these are unusual types, not TEM and SHV variants [3, 4]. Unfortunately, these enzymes may not be easily detectable in the laboratory and thus lead to treatment failure with what may be deemed to be an appropriate therapy.

Infection with ESBL-producing bacteria is usually hospital-acquired, especially in the ICU settings as well as in nursing homes [5]. The common types of infection are urinary tract infection, peritonitis, and intra-abdominal sepsis. This is because the normal habitat of the Enterobacteriaceae is the gut from where the bacteria can easily reach these sites. However, they can colonize the upper respiratory tract and the skin of hospitalized patients, resulting in hospital-acquired pneumonia and central line-related bacteremia. In hospitalized patients who had undergone neurosurgical procedures, ESBL-producing organisms may cause meningitis. The major problems with these infections are the associated limitation of antibiotic therapeutic options, significantly longer hospital stays [6, 7], higher cost of management [7] and clinical failure [8]. These important problems make it prudent to be able to detect ESBLs in the clinical laboratory for optimal therapy and infection control intervention. The aim of the study was to compare the ability of VITEK 2 (bioMérieux, Marcy-l'Etoile, France) and E test (AB Biodisk, Solna, Sweden) in detecting ESBL production in multiresistant isolates of Enterobacteriaceae and *P. aeruginosa* and to determine the prevalence of ESBL-producer strains in our hospital.

Materials and Methods

Bacterial Isolates

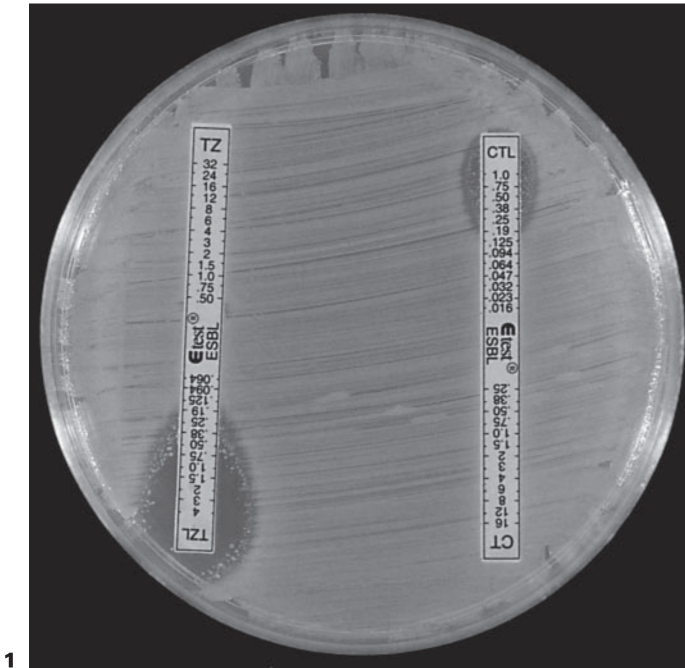
A total of 3,592 clinically significant bacterial strains belonging to the family Enterobacteriaceae, that were isolated from patients seen and treated at the Mubarak Al-Kabeer Hospital, Kuwait, between January and December 2003, were studied for ESBL production. The main criterion for selection of strains for comparative study was that they were consecutive isolates clinically relevant to patients' condition enough to warrant giving antibiotic therapy. Duplicate isolates from the same patient were not included in the study.

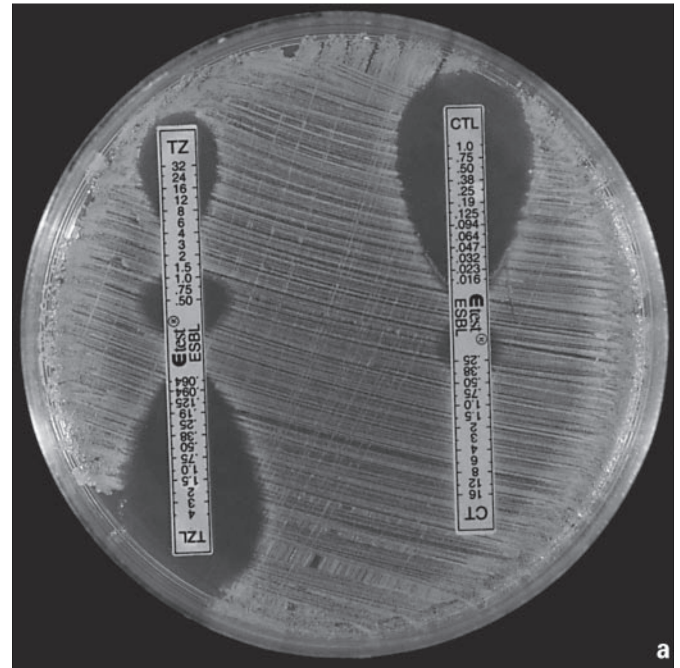
VITEK 2 Analysis

VITEK cards (ID-GNI) were used for identification of all gram-negative bacilli (GNB). Antimicrobial susceptibility test was done by using two types of VITEK cards: VITEK card AST-N022 for antimicrobial susceptibility of non-lactose-fermenting, oxidase-positive GNB, and AST-N020 for lactose-fermenting and non-lactose-fermenting, oxidase-negative GNB. They were inoculated according to the manufacturer's instruction. The results were interpreted by using software version VTK-R01.02, an advanced expert system (AES) [2, 9–11]. The AES is based on over 2,000 phenotypes and 20,000 MIC distributions, which have been derived from published literature, internal data generated at bioMérieux, and outside experts. Following the identification and antimicrobial susceptibility testing of the bacterial isolates, the AES software then searches for the MIC distributions in its knowledge base to ascertain if the result is consistent with any of the phenotypes established for a particular bacterium in order to establish a biological validation. This comparison allows the AES to determine if the identification is consistent with the susceptibility pattern and if the MICs are consistent with a specific phenotype. At the end of each run, a printout containing the MIC values of the antibiotics with ESBL status of each isolate was obtained and recorded.

E Test ESBL

Both cefotaxime/cefotaxime combined with clavulanic acid strip and ceftazidime/ceftazidime with clavulanic acid strip were used. E test ESBL was performed according to the manufacturer's instruction. Briefly, an overnight culture of the bacteria diluted to a 0.5 McFarland turbidity standard was used to inoculate Mueller-Hinton agar plate (Oxoid, Basingstoke, UK). After drying, the E test strips were applied on the plates and incubated overnight at 37°C. The MICs on both ends were read on the intersection of the inhibition ellipse and the E test-strip edge. *E. coli* ATCC 35218, which produces TEM-1-ß-lactamase (non-ESBL), was used as negative control. ESBL-positive control strain of *K. pneumoniae* ATCC 700603 and a local strain of ESBL-positive *P. aeruginosa,* producing a VEB-1-like ESBL [4], were used as positive controls. They were tested with each run.





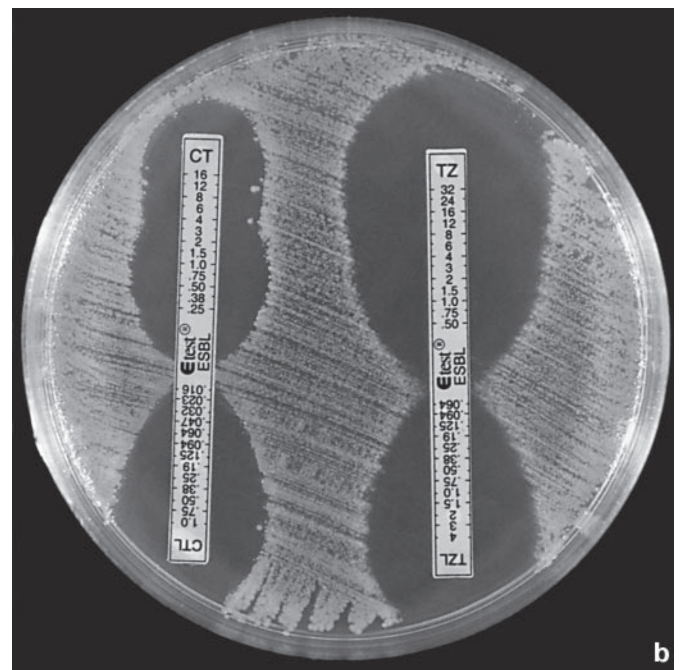


Fig. 1. A clear-cut E test ESBL positivity in *E. coli* showing MIC of CT/CTL = 16/0.23, ratio >8 and MIC of TZ/TZL = 32/0.19, ratio >8. CT = Cefotaxime; CTL = cefotaxime combined with clavulanic acid; TZ = ceftazidime; TZL = ceftazidime combined with clavulanic acid.

Fig. 2. a E test ESBL-positive isolate showing a rounded phantom inhibition zone below TZ and CT. The rounded phantom inhibition zone is indicative of ESBL production. **b** E test ESBL production indicated by a deformation of the CT and TZ inhibition ellipse.

Results

The synergic activity of clavulanic acid (L) with both ceftazidime (TZ) and cefotaxime (CT) was confirmed by means of two different E test strips containing ceftazidime and cefotaxime with or without clavulanate. In accordance with the manufacturer's interpretative criteria, the presence of ESBL was confirmed when the MIC of either cefotaxime or ceftazidime is reduced by ≥ 3 $\log_2$ dilutions in the presence of clavulanic acid. In other words, an isolate is considered as ESBL-positive when the MIC of TZ ≥ 1 μg/ml and the ratio of TZ/TZL ≥ 8 μg/ml or MIC of CT ≥ 0.5 μg/ml and the ratio of CT/CTL ≥ 8 (fig. 1). In addition, a strain was considered ESBL-posi-

Table 1. The prevalence of ESBL-producing bacterial isolates in Mubarak Hospital

Bacteria	Total number of isolates	Number (%) of ESBL-positive isolates by	
		E test	VITEK 2
E. coli	2,107	119 (5.6)	137 (6.5)
P. aeruginosa	536	0 (0)	35 (6.5)
K. pneumoniae	509	58 (11.4)	62 (12.2)
Enterobacter spp.	134	4 (3.0)	16 (12)
Proteus spp.	93	1 (1.1)	1 (1.1)
Citrobacter spp.	63	2 (3.2)	10 (15.9)
Morganella spp.	50	1 (2.0)	2 (4.0)
Stenotrophomonas	36	0 (0)	1 (2.8)
Total	3,529	185 (5.2)	264 (7.5)

Table 2. Distribution of ESBL-positive isolates determined by VITEK 2 by source

Specimen	*E. coli*	*Klebsiella*	*Pseudomonas*	*Enterobacter*	Others
Urine	103	43	14	12	8
Blood	6	7	2	1	0
Wound	18	6	9	2	3
Cerebrospinal fluid	1	0	0	0	0
Respiratory secretions	3	5	7	0	1
Others	6	1	3	1	2
Total	137	62	35	16	14

Table 3. Distribution of ESBL-positive isolates of Enterobacteriaceae determined by E test and VITEK 2

Bacterial isolate	E test			VITEK 2
	positive	negative	indeterminate	
E. coli	119	9	9	137
K. pneumoniae	58	1	3	62
P. aeruginosa	0	0	35	35
Enterobacter spp.	4	0	12	16
Citrobacter spp.	2	2	6	10
Others	2	0	2	4
Total	185	12	67	264

tive if a phantom zone or a deformity of ceftazidime and cefotaxime zone could be observed independent of the ratios or MICs (fig. 2). The outcome of the result was indeterminate when both MICs were outside the test range of the test device or when the result of one strip was negative and the result of the other strip was indeterminate, for example, when the TZ/TZL ratio was >32/>4 (indeterminate) or CT/CTL was ESBL-negative (6/>1) and TZ/TL indeterminate.

The analysis of the data showed that there were discrepancies between VITEK 2 and E test detection of ESBLs. Of 3,529 clinically significant isolates, 264 (7.5%) and 185 (5.2%) were ESBL producers by VITEK 2 and E test, respectively. All those positive by the E test were also positive by the VITEK 2 system. The prevalence of ESBL-producing bacterial isolates is shown in table 1. The prevalent ESBL producers identified by the VITEK 2 versus E test, respectively, were as follows: *Citrobacter* spp. (15.9 versus 3.2% of 63 isolates), *K. pneumoniae* (12.2 versus 11.4% of 509), *Enterobacter* spp. (12 versus 3% of 134), *E. coli* (6.5 versus 5.6% of 2,107), *P. aeruginosa* (6.5 versus 0% of 536), and *Morganella* spp. (4 versus 2% of 50).

The distribution of the 264 ESBL-positive isolates detected by the VITEK 2 according to source is shown in table 2. Those positive by the E test detection method were from the same sources as the VITEK 2. The most common infection that yielded ESBL-producing bacteria was urinary tract infections, accounting for 180 (68.2%) out of 264. This was followed by wound infections, 38 (14.4%), bacteremia, 16 (6.1%), respiratory secretions, 16 (6.1%), and others 13 (4.9%), excluding 1 case of meningitis (0.4%). The overall commonest ESBL producers were *E. coli* and *K. pneumoniae*, representing 137 (51.9%) and 62 (23.5%) of the 264 isolates, respectively. The majority of the blood isolates were *K. pneumoniae* (7/16; 43.8%) and *E. coli* (6/16; 37.5). One of the ESBL-producing *E. coli* isolates was isolated from cerebrospinal fluid of the patient with meningitis. In patients with urinary tract infection, *E. coli* (103/180; 57.2%) was the predominant ESBL producer, followed, in decreasing prevalence, by *Klebsiella* (43/180; 23.9%), *P. aeruginosa* (14/180; 7.8%) and *Enterobacter* spp. (12/180; 6.7%).

The numbers of the ESBL-producing bacterial isolates detected by E test and VITEK 2 are shown in table 3. A total of 185 (70.1%) isolates were positive by the E test; the remaining 67 (25.4%) and 12 (4.6%) were indeterminate and negative, respectively. All of the *P. aeruginosa* strains gave indeterminate results by E test, followed by 12 (17.9%) of *Enterobacter*, 9 (13.4%) of *E. coli*, 6 (9%) of *Citrobacter* and 3 (4.5%) of *Klebsiella* sp.

Discussion

ESBLs have been found in a wide range of gram-negative bacteria; but the vast majority of strains producing these enzymes belong to the family Enterobacteriaceae. In many hospitals, *K. pneumoniae* remains the major ESBL producer [1]. However, *E. coli* is another important bacterium, which has a high representation among ESBL producers circulating in the hospital environment [1, 12, 13]. ESBL has also been detected in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes* [14]. Sporadic isolates of ESBL-producing *Salmonella* spp. or nosocomial outbreaks caused by these organisms have been identified in several countries in Latin America, Africa, Europe and Asia [15–18]. There are very scanty reports of non-Enterobacteriaceae ESBL producers, which are considered rare, with *P. aeruginosa* being the most important among them [3, 4]. The result of our present study shows that the frequency of ESBL producers in hospitals varies. In our hospital, the overall prevalence was 7.5%. A recent study in Brooklyn, N.Y. showed that ESBLs were produced by 17.2% of selected members of the Enterobacteriaceae *(K. pneumoniae*, *E. coli*, and *Proteus mirabilis)* [19], in contrast to a wider French investigation, which revealed an overall prevalence of 3.2% of ESBL-producing Enterobacteriaceae [20]. The prevalence rate in our hospital is similar to that in the report of a wide Italian survey obtained from 10 medical centers, which indicated that 6.3% of Enterobacteriaceae harbor ESBL genes [21]. In that Italian study, the prevalence of ESBL-positive *K. pneumoniae, E. coli* and *P. mirabilis* was 20, 1.2 and 16.3%, respectively, which contrasts with our findings on these individual pathogens. The prevalence of ESBL-producing *Klebsiella* and *Proteus* was considerably lower and *E. coli* higher in our study compared to that of Spanu et al. [21]. A report from some institutions in the Netherlands indicated that the prevalence of these organisms producing ESBL was less than 1% [22], while another, a multicenter ICU study in the same country, showed a contrasting higher prevalence of 16% for *Klebsiella* [23]. In this present study, the prevalence of ESBL-producing *Enterobacter* of 12% is almost a quarter of the 46% rate reported by Tzelepi et al. [14]. However, the prevalence of ESBL in our *Citrobacter* spp. was comparable to that found during an outbreak of *Citrobacter freundii* infections in Poland [24].

There is currently a great need for a reliable test to detect ESBLs in clinical isolates of Enterobacteriaceae. The test needs to be practically feasible for routine use in the clinical laboratory. E test, in our study, failed to detect all ESBL-positive *P. aeruginosa* and nearly 30% of other gram-negative organisms in comparison to VITEK 2. Although some studies have demonstrated reliable results with E test [25–27], others did not [22, 23, 28]. Even Leverstein-van Hall et al. [27], who stated that E test gave an accurate result, reported that there were some limitations with the test, which included the indeterminate results in 5% and inability to differentiate between chromosomal K1 (KOXY) hyperproduction and ESBLs. Our ESBL detection rate by E test of 70%, using the same manufacturer's recommendations, is slightly discordant with the 81% rate obtained by Vercauteren et al. [23], a rate that increased to 88% when the recommendation of Cormican et al. [28] was adopted, using a breakpoint of 5 instead of 8. It is noteworthy that our study recorded a 25.3% indeterminate result with E test and a false-negative result in 4.5%. Some other limitations of the E test ESBL method include the fact that a well-trained and experienced technical staff is needed, at least for the interpretation at all times and introduction of additional guidelines for reading and interpretation, which may increase the burden of the test.

The advantage of using ceftazidime/ceftazidime plus clavulanate has been reported; Stobberingh et al. [22] found fewer false-positive results when using ceftazidime/ceftazidime plus clavulanic acid than with cefotaxime/cefotaxime plus clavulanic acid. No such disparity was found in our study. Although the E test is relatively easy to perform, it is, however, expensive and there is difficulty in reading the mutant colonies along the zone border and inside the inhibition zone. In addition, this test in our study missed detection of all ESBL strains of *Pseudomonas* and about 30% of other gram-negative organisms. Therefore, it may not be ideal for routine use in our clinical laboratory.

The VITEK 2 ESBL test, on the other hand, proved to be more reliable and easy to perform without any subjective interpretation of the results. It was able to detect ESBLs in clinical strains of *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp. as well as *Pseudomonas* spp. It is plausible that there is the possibility of false-positive results with the VITEK 2. However, with the addition of new ESBLs to the database used in developing the AES in the improved VITEK 2, the chances of recording false-positive results is considered low.

The high level of interest in developing tests for the detection of ESBL-producing Enterobacteriaceae under-

scores the great need for such tests. Until such tests become available, many hospitals will continue to run the risk of missing some potentially problematic bacteria with consequent dissemination of resistant strains. Conceivably, patients will continue to be at risk of becoming infected with strains that will not respond to what appears to be an appropriate antibiotic therapy. Since patients most likely to become infected with ESBL-producing Enterobacteriaceae are those with prolonged hospital stays, such as patients in the ICU, and those who have invasive procedures, any delay in detection of this often hidden mechanism of resistance could have serious consequences [29, 30], including treatment failure [8, 30, 31].

Conclusion

It is worthy of note to recognize the fact that there are more than 100 ESBLs known worldwide as of today. Since this is subject to rapid evolution and is involved in dynamic epidemiology [32], it is conceivable that the results of our study or any other study might not be applicable to all laboratories in other geographical locations throughout the world at this time, especially if the old VITEK 2 with limited ESBL database is used. This study showed a relatively high prevalence of clinically significant ESBL producers among the Enterobacteriaceae and *P. aeruginosa* and VITEK 2 appears to be superior to E test in detecting ESBL.

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