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# Comparison of Phenotypic Screening Methods for Detection of Extended-spectrum β-lactamase Producers among Pediatrics with Bloodstream Infections in a Saudi Hospital

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**In the present investigation, the author aimed first at detection of Gram-negative isolates harboring ESBL in pediatrics with bloodstream infection at Madinah Maternity and Children Hospital, Saudi Arabia. The second aim was to predict the most suitable antibiotics against them. ESBL detection was done using different screening methods as disk diffusion test, antibiotic-containing agars and double-disk synergy test as initial screening tests; Phoenix system as a commercial method; and cephalosporin/clavulanate combination disks as a confirmation test. Of the 112 Gram-negative isolates tested, 22 (19.6%) were identified as ESBL producers based on the results of the confirmation test. 45.5% of them were *K. pneumonia.* ESBL producers showed the highest susceptibility to ciprofloxacin, piperacillin/tazobactam and meropenem. On contrary, they were highly resistant to aztreonam, ceftazidime and cefotaxime. The occurrence of ESBL producers was higher in ICUs neonates. 18 isolates (16%) revealed presence of inducible AmpC β-lactamases by disk antagonism test. It is essential to report ESBL production along with the routine sensitivity reporting to help the clinicians prescribe proper antibiotics.**

**Key words:** Bloodstream infections, pediatrics, Gram-negative bacteria, extended-spectrum β-lactamase, Saudi Arabia, phenotypic screening.

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Gram-negative bacteria are still among the most important agents involved in pediatric bloodstream infection (BSI), responsible for approximately 24% to 50% of the cases[1,2,3].

**Mortality and morbidity from BSI are greater when caused by antimicrobial resistant bacteria[4]**

β-lactam antibiotics are the cornerstone of most of the severe bacterial infections[5]. They are generally characterized by their favorable safety and tolerability profiles as well as their broad-spectrum of activity. Hence, they are typically used as first-line therapy in different types of infections[6]. However, the use of β-lactam antibiotics could be limited by the spreading of resistant bacteria in the society[7,8].

There are various mechanisms of bacterial resistance to β-lactams, the most important is the production of β-lactamases, which hydrolyze the β-lactam ring of penicillins, cephalosporins and related antimicrobial drugs, rendering them inactive. There are dozens of β-lactamases that vary in substrate specificity.

During the past decade, drug resistance in *Enterobacteriaceae* has increased dramatically worldwide. This increase has bee caused mainly by an increased prevalence of extended spectrum

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β-lactamase (ESBL)-producing enterobacteriaceae[9]. Thus, the identification of enterobacteriaceae producing ESBL is imperative for clinical microbiology laboratories[10].

The likely consequence of not seeking ESBL enzymes for treatment purposes is that some laboratories will not seek them at all, leading to a loss of critical infection control information[11].

Detection of ESBLs can be done with phenotypic or genotypic tests. The phenotypic tests are routinely used in clinical diagnostic laboratories, whereas the genotypic tests are mainly used in reference or research laboratories[12].

In the present investigation, the authors aimed first at comparison of different phenotypic methods used in detection of Gram-negative isolates harboring ESBL in pediatric patients with BSI at Madinah Maternity and Children's Hospital (MMCH), Al-Madinah, Saudi Arabia, and predict the most suitable antibiotics against ESBL organisms. The second aim of the study was to determine the occurrence of AmpC enzyme-harboring Gram-negative clinical isolates.

## MATERIALS AND METHODS

### Bacterial isolates

During one year period from July 2009 to June 2010, 261 Gram-negative bacteria were isolated from the pediatrics suffering from BSI in the different wards of Madinah Maternity and Children's Hospital (MMCH). The age range of patients was 1-14 year; male to female ratio was 1.2 : 1. Of them, 112 isolates were tested for ESBL production. They were comprising: *K. pneumoniae* (n=28), *S. marcescens* (n=25), *P. aeruginosa* (n=18), *Enterobacter* spp. (n=13), *E. coli* (n=10), *Acinetobacter* spp. (n=4), *Stenotrophomonas maltophilia* (n=3), *Salmonella* spp. (n=3), *Chryseomeningosept* spp. (n=3), *Citrobacter* spp. (n=2), *K. oxytoca* (n=2) and *Moraxella* spp. (n=1).

### Detection of ESBL producers

### Initial screening tests

Three methods were used for initially screening of ESBL production among the studied isolates:

### Disk diffusion test (DDT)

The performance of the test isolates was assessed using cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg) and cefpodoxime (10 µg) placed on inoculated plates of Mueller-Hinton agar. The bacterial suspension was adjusted to be equivalent to 0.5 McFarland standards and the test was done according to the CLSI[13] recommendations. BSI isolates showed inhibition zone size of cefpodoxime zone ≤ 17 mm, ceftazidime zone ≤ 22 mm, aztreonam zone ≤ 27 mm, cefotaxime zone ≤ 27 mm and ceftriaxone zone ≤ 25 mm, were identified as potential ESBL producers.

### Antibitic-containing agars (ACA)

Two MacConkey's agars were used; the first medium was (Mac X medium) consisting of MacConkey's agar supplemented with 1.0 mg/l cefotaxime sodium (Fabriqué par, France); while the second medium was (Mac Z medium) consisting of MacConkey's agar supplemented with 1.0 mg/l ceftazidime anhydrous (Fabriqué par, France)[14].

### Double-disk synergy test (DDST)

It was performed by a standard disk diffusion assay on Mueller-Hinton agar. Disks containing cefotaxime, ceftazidime, cefepime, ceftriaxone and aztreonam (30 µg each) were placed at distances 30 mm from center to center, around a disk containing amoxicillin/clavulanate (20 µg/10 µg). The isolates showed enhancement of the inhibition zone toward the amoxicillin/clavulanate disk was considered suggestive of ESBL production[15].

### Automated method (Phoenix ESBL test)

The Phoenix ESBL test used five wells containing the fixed concentrations of the following drugs: cefpodoxime, ceftazidime, ceftazidime plus clavulanic acid (CA), cefotaxime plus CA and ceftriaxone plus CA. Before inoculating panels, the pure isolates was suspended in Phoenix ID broth to obtain a 0.5 McFarland suspension using the BD PhoenixSpec™ nephelometer and then tested according to the manufacturer provided protocol. In order to ensure appropriate procedure and acceptable performance of the system, *E. coli* ATCC™ 25922 was used as ESBL negative control; and *K. pneumoniae* ATCC™ 700603 was used as ESBL positive reference strain[13].

### Phenotypic confirmatory test by cephalosporin/clavulanate combination disks method (CCCD)

Confirmation of the ESBL phenotype was carried out using the cephalosporin/clavulanate combination disks performed[16]. The test depends on comparing the inhibition zones given by cefpodoxime (CPD) (10 µg) and cefpodoxime/clavulanate (CD01) (10 plus 1 µg) disks (Oxoid, UK). A difference of ≥ 5 mm between the zones of the CD01 (10 plus 1µg) and CPD (10 µg) disks indicates ESBL production[17].

**Detection of inducible AmpC β -lactamases by disk antagonism test (DAT)**

To detect inducible resistance to third-generation cephalosporins, cefoxitin (30 µg) as a β-lactamase induction agent; and cefotaxime (30 µg) and ceftazidime (30 µg) as the third-generation cephalosporin reporter agents were used. β-lactamase inducibility was recognized by blunting of the cefotaxime and ceftazidime zone adjacent to the cefoxitin disk[18].

**Determination of MIC for ESBL producers**

In addition, the MIC to cefotaxime, ceftriaxone, ceftazidime, cefoxitin, cefepime, aztreonam, imipenem, meropenem, piperacillin/tazobactam, gentamicin, amikacin and ciprofloxacin was determined by BD Phoenix™ automated microbiology system (BD Diagnostic Systems, Sparks, MD) by using BD Phoenix™ NMIC/ID panels according to manufacturers' specifications.

**Statistical methods**

Sensitivity, specificity, prevalence of ESBL positive predictive value (PPV) and negative predictive value (NPV) of the initial screening tests were calculated compared to the confirmatory method (CCCD) as the following:

Sensitivity = True positives/True positives + False positives= a/a+c

Specificity= True negatives /True negatives + False negatives= d/b+d

Prevalence of ESBL producers among the studied isolates = a+c/ a+b+c+d X 100

PPV = a/a+b X 100, NPV= d/c+d X100

## RESULTS

**Detection of ESBL producers (Table 1)**

**DDT**

Out of the 112 Gram-negative isolates tested, 45 (40%) were resistant to more than two or all the tested antibiotics).

**ACA**

The studied Gram-negative isolates were grown on the control MacConkey, but were

**Table 1.** Different phenotypic methods for detection of ESBL producers among BSI Gram-negative isolates understudy (n= 112)

| Microbial isolates (No.) | No. of ESBL producers by: | | | | | |
|---|---|---|---|---|---|---|
| | DDT | DDST | Mae X | Mac z | Phoenic | CCCD |
| *K. pneumoniae* (28) | 10 | 17 | 5 | 5 | 10 | 10 |
| *K. oxytoca* (2) | 1 | 1 | 1 | 1 | 1 | 1 |
| *E. coli* (10) | 1 | 2 | 1 | 0 | 1 | 1 |
| *P. aeruginosa* (18) | 18 | 0 | 7 | 6 | 18 | 2 |
| *E. cloacae* (9) | 0 | 3 | 0 | 0 | 0 | 0 |
| *S. marcescens* (25) | 3 | 8 | 0 | 1 | 3 | 2 |
| *E. agglomerans* (1) | 0 | 0 | 0 | 0 | 0 | 0 |
| *E. gergoviae* (1) | 0 | 1 | 0 | 0 | 0 | 0 |
| *E. aerogenes* (2) | 0 | 0 | 0 | 0 | 0 | 0 |
| *Salmonella* spp. (3) | 2 | 2 | 1 | 2 | 2 | 2 |
| *C . freundii* (1) | 1 | 0 | 1 | 0 | 1 | 1 |
| *C . koseri* (1) | 0 | 0 | 0 | 0 | 0 | 0 |
| *Acinetobacter* spp. (4) | 3 | 0 | 1 | 0 | 3 | 0 |
| *Chryseo meningosept* (3) | 3 | 0 | 0 | 0 | 3 | 3 |
| *S. maltophilia* (3) | 3 | 3 | 0 | 0 | 3 | 0 |
| *Moraxella* spp. (1) | 0 | 1 | 0 | 0 | 0 | 0 |
| Total (112) | 45 | 38 | 17 | 15 | 45 | 22 |

*E*SBL= Extended-spectrum β-lactamase, DDT= Disk diffusion test, DDST= Double-disk synergy test, Mac X= MacConkey's agar supplemented with cefotaxime sodium, Mac Z= MacConkey's agar supplemented with ceftazidime anhydrous, CCCD= Cephalosporin/clavulanate combination disks.

**Table 2.** MIC of the antibaterial agents used against the ESBL producers among BSI Gram-negative isolates understudy

| | Total No. (%) | | MIC (Antibacterial resistance rates No.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *K. pneumoniae* (n= 28) | *K. oxytoca* (n= 2) | *E. coli* (n= 10) | *S. marcescensn* (n= 25) | *P. aeruginosa* n= (18) | *Salmonella* spp. (n= 3) | *C. meningosepticum* (n= 3) | *Citrobacter* spp. (n= 2) |
| | Non -ESBL | ESBL | ESBL PRODUCERS | | | | | | | |
| | | | Beta-lactams | | | | | | | |
| CTX | 16 (23.2) | 18 (81.8) | > 32 (10) | > 32 (1) | > 32 (1) | 0 (0) | > 32 (2) | 0 (0) | > 32 (3) | > 32 (1) |
| CAZ | 1 (1.4) | 19 (86.4) | > 16 (10) | > 16 (1) | > 16 (1) | 0 (0) | > 16 (1) | > 16 (2) | > 16 (3) | > 16 (1) |
| ATM | 7 (10) | 20 (90.9) | > 16 (10) | > 16 (1) | > 16 (1) | > 16 (2) | 0 (0) | > 16 (2) | > 16 (3) | > 16 (1) |
| CRO | 23 (33) | 17 (77.3) | | | | | | | | |
| FEP | 1 (1.4) | 17 (77.3) | > 16 (10) | > 16 (1) | > 16 (1) | 0 (0) | 0 (0) | > 16 (1) | > 16 (3) | > 16 (1) |
| FOX | 44 (63.8) | 12 (54.5) | > 16 (2) | 0 (0) | 0 (0) | > 16 (2) | > 16 (2) | > 16 (2) | > 16 (3) | > 16 (1) |
| IMI | 9 (13) | 4 (18.2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | > 8 (1) | 0 (0) | > 8 (3) | 0 (0) |
| MEM | 3 (4.3) | 3 (13.6) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | > 8 (3) | 0 (0) |
| | | | β-lactams/β-lactamase inhibitors | | | | | | | |
| TZP | 2 (2.9) | 3 (13.6) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | > 64/4 (3) | 0 (0) |
| | | | non β-lactams | | | | | | | |
| GM | 6 (8.7) | 12 (54.5) | > 8 (6) | > 8 (1) | 0 (0) | 0 (0) | 0 (0) | > 8 (2) | > 8 (3) | 0 (0) |
| AK | 5 (7.2) | 6 (27.3) | 0 (0) | > 32 (1) | 0 (0) | 0 (0) | 0 (0) | > 32 (2) | > 32 (3) | 0 (0) |
| CIP | 3 (4.3) | 2 (9.1) | > 2 (2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

CTX= Cefotaxime, CAZ= Ceftazidime, ATM= Aztreonam, CRO = Ceftriaxone, FEP= Cefepime, FOX= Cefoxitin, IMI= Imipenem, MEM= Meropenem, TZP= Piperacillin/tazobactam, GM= Gentamicin, Ak= Amikacin, CIP= Ciprofloxacin.

inhibited from growing on the two antibiotic-containing agars, except 23 isolates (20.5%) that grew on at least one of the selective media after 18 to 24 h of incubation.

**DDST**

Synergy between CA and at least one of the tested β-lactams was observed for 38 isolates (33.9%) of the total isolates tested.

**CCCD**

22 isolates (19.6%) were defined as ESBL producers. ESBL producer gave zones at least 5 mm larger with the CD01 disk than with the CPD disk alone. None of the rest 90 ESBL negative isolates gave a difference in zone diameter of more than 1 mm between the two disk types.

Out of the 22 ESBL producers, *K. pneumoniae* was detected in 10/22, 45.5%; *Chryseobacterium meningosepticum* 13.6% (3/22), *S. marcescens* 9.1% (2/22), *P. aeruginosa* 9.1% (2/22), *Salmonella* spp. 9.1% (2/22), and 1/22, 4.5% of each of C. *freundii*, *K. oxytoca* and *E. coli*.

Most ESBL producers were detected by cefpodoxime (n=22/22, 100%) followed by aztreonam (n=20/22, 90.9%), ceftazidime (n=19/22, 86.4%), cefotaxime (n=18/22, 81.8%) and ceftriaxone (n=17/22, 77.3%).

Sensitivities, Specificities, PPV, NPV of the initial screening tests were calculated compared to the confirmatory method.

Sensitivity (%) of DDT, ACA, DDST and phoenix was 100, 72.73, 45.45, 100, respectively.

Specificity (%) of DDT, ACA, DDST and phoenix was 74.44, 92.22, 68.89, 74.44, respectively.

PPV (%) of DDT, ACA, DDST and phoenix was 48.89, 69.57, 26.32, 48.89, respectively.

NPV (%) of DDT, ACA, DDST and phoenix was 100, 93.26, 83.78, 100, respectively.

**Occurrence of inducible AmpC β-lactamases**

18 BSI isolates (16%) (14 *P. aeruginosa*, 3 *S. maltophilia* and 1 *Enterobacter* spp.) were found to be AmpC producers. Simultaneous occurrence of ESBL and inducible AmpC enzymes was noted in one isolate of *P. aeruginosa*.

**Phoenix MIC test**

MIC of all antibiotics is presented in Table 2. As expected, the ESBL producers showed a higher resistance rates than the non ESBL producers towards all the antibacterial agents tested, with the exception of cefoxitin. The non ESBL producers showed a higher resistance with cefoxitin (63.8%). On the other hand, ESBL producers showed a higher resistance with cefpodoxime (100%), aztreonam (90.9%) and ceftazidime (86.4%).

Out of the 22 cases infected with ESBL producers: 12 ( 54.5%) of age ≤ 1 month; and 14 (63.64%) with ≥ 1 month hospital stay. Mortality rate among them was 36.36% (8/22); and all of them were from ICUs (16 from NICU & 6 from PICU).

## DISCUSSION

Antibiotic resistance is now considered a global health problem that increases the morbidity, mortality and costs of treating infectious diseases[19]. This issue is particularly serious in developing countries where bacterial infections remain the major causes of morbidity and mortality, especially in childhood[20]. β-lactamase producers are emerging threat and cause of concern for the clinicians, as it results in the resistance to penicillin, cephalosporins and limits therapeutic options. Screening techniques should be performed routinely to detect them so that suitable antimicrobial therapy can be instituted[21].

There are many published reports on ESBL-producing microorganisms in developed and developing countries[22-26]. Moreover, from Saudi Arabia, documented evidence of the spread of multiresistant ESBL-producers into the community were reported[27].

Since ESBL-producing strains prevalence can vary greatly from one site to another and even over time for a given site, so regional and local estimates are probably more useful to clinical decision-making than are more global assessments[28].

Detection of organisms producing an ESBL is not always easy with routine susceptibility testing[29]. There is a need for a sensitive and simple screening procedure to enhance the detection of ESBL-producers[30]. An initial screening test to facilitate the detection of ESBL-producing bacteria in a clinical setting is important not only for guiding treatment, but also for early implementation of appropriate infection control measures[14]. Therefore, we compared different ESBL detection methods.

Detection of ESBL producers among the selected Gram-negative isolates in this setting was done by preliminary screening that depends on reduced susceptibility to one or more of (cefpodoxime, ceftazidime, aztreonam, cefotaxime and ceftriaxone). 45 isolates (40%) were considered as potential producers of ESBLs by DDT. DDT revealed an additional 23 isolates that were positive for ESBL compared to the confirmatory test. This suggests that those strains may produce an ESBL enzyme and a AmpC-enzyme.

The sensitivity of screening for ESBL can vary depending on the type of antimicrobial agent tested[31]. Ceftazidime and cefpodoxime were chosen because they are the best third-generation cephalosporin substrates for most TEM- an SHV-derived ESBLs [32,33]. Cefpodoxime in this study showed the highest activity (100%) in ESBL detection while ceftriaxone had the least activity (77.3%) [34].

In our setting, only 86.4% of ESBL producers were detected by ceftazidime. Although was the best single indicator antibiotic for ESBL production, all strains producing TEM-4, TEM-12, SHV-2, SHV-3, or SHV-5 remained undetected by it[35].

In Wilson & McCabe[14] study, from Scotland, on ICU patients, the combined use of MacConkey screening agars supplemented with either ceftazidime or cefoxatime enabled 100% detection of known ESBL producers. Variations in the efficiency of detection of ESBL-producing organisms with a single selective antibiotic have been reported[36]. It is therefore unlikely that a single selective medium will detect all known ESBL producers[37]. That is the reason we used two antibiotics in ACA method.

Sensitivity of ACA method was 72.73%. This may be explained by the fact that the selective plates will allow the growth of any organism that has resistance to the antibiotic incorporated in the agar, but a compensating benefit is that the agars may also isolate organisms that hyperproduce AmpC enzymes[14].

Regarding the DDST, it is the most widely used test due to its simplicity and ease of interpretation[38]. The sensitivity of the DDST range from 79% to 96% in different studies[39].

The low sensitivity (45.45%) and specificity (68.89%) of DDST may be explained by the following reasons. First, DDST is not a standardized procedure[39]. Second, the sensitivity of DDST varies with the distance between the disks[38,40]. Third, sensitivity may be reduced when ESBL activity is very low leading to wide inhibition zones around the cephalosporin and aztreonam[35]. Fourth, AmpC producers and most hyperproducers of K1 enzyme give negative results with all the three cephalosporins[40]. Fifth, false negative results have been observed with isolates harboring SHV-2, SHV-3 and TEM-12 [35,41].

We have detected 100% sensitivity and 74.44% specificity of Phoenix ESBL test compared to DDST. In accordance[42] found that Phoenix ESBL test provided accurate results when tested against the DDST, with 100% sensitivity and 98.9% specificity.

CCCD method confirmed ESBL production. It uses cefpodoxime (10 µg) as a partner agent for CA (1 µg) to detect ESBL-positive Klebsiella strains with 100% sensitivity and specificity[17]. This method makes it possible to distinguish isolates with AmpC or K1 enzymes, whose cefpodoxime inhibition zone is not enhanced by CA. Cefpodoxime has indeed been shown as the best general substrate to screen for all types of ESBLs presently found in clinical specimens[43].

The various susceptibility testing methods differed in their ability to detect cephalosporin resistance in the ESBL-producing strains[10]. In analysis of the results of the different initial ESBL detection methods, both DDT and Phoenix system detected the same 45 ESBL producers.

AmpC enzymes are chromosomal and inducible in most *Enterobacter* spp., *C. freundii*, *Serratia* spp., *M. morganii*, *Providencia* spp. and *P. aeruginosa*[44]. AmpC β-lactamases are class C or group I cephalosporinases that confer resistance to a wide variety of β-lactam antibiotics including alpha methoxy β-lactams such as cefoxitin, narrow and broad-spectrum cephalosporins, aztreonam. They are also poorly inhibited by β-lactamase inhibitors such as clavulanic acid[45]. In this work, most AmpC producers (78%) were *P. aeruginosa* and none of the *E.coli, Klebsiella* or *Acinetobacter* showed inducible AmpC positive. Mohamudha *et al.*[46] detected a maximum number of 42.8% of *Pseudomonas* spp. were inducible AmpC producers followed by *Enterobacter* spp. 41.6%; with none of the *E.coli, Klebsiella* or *Acinetobacter* showed inducible AmpC positive.

ESBL producers vary in their capacities to hydrolyze specific β-lactam drugs because of their different enzymatic properties[47]. Confounding factors, such as the production of different β-lactamases by the same organism, can also lead to erroneous phenotypic conclusions[42]. Clinical isolates producing as many as five distinct β-lactamases have been identified. Moreover, the phenotypic response is the result not only of the hydrolytic affinity of a given enzyme for its β-lactam substrate but also of the amount of enzyme produced[47].

Treatment of infections caused by ESBL producers is complicated not only by resistance to extended-spectrum cephalosporins, but also because many ESBL genes are on large plasmids containing genes which are transferable between one bacterium to another which also encode resistance to many other antibiotics including aminoglycosides, chloramphenicol, sulfonamides and tetracycline antibiotics[48]. Generally, MDR has been reported among ESBL-producing organisms[49].

Different phenotypic characteristics among ESBL producers pushed us to undergo antibiotic sensitivity testing. Ciprofloxacin had the best activity with the lowest resistance rate (9.1%) against ESBL producers in the present work. Similarly[50], reported a lower ciproflaxin resistance rate of ESBL-producing isolates (11.3%). In Iran, resistance to ciprofloxacin was found among 32% of the ESBL-producing *K. pneumoniae* strains[51]. Furthermore, piperacillin/tazobactam had a low resistance rate (13.6%) against ESBL organisms. On contrary, previous studies[52,53] had documented treatment failures due to the use of β-lactam/β-lactamase inhibitor combinations for infections caused by ESBL producers.

Different studies have indicated a relationship between antibiotic usage and resistance and we speculate that similar scenario may be at play in our setting as there is a high level of antibiotic prescription and misuse occurring in Saudi Arabia.

Based on our *in vitro* findings, carbapenems (meropenem and imipenem) had a high activity against the ESBL-producers, similar to[54,55]. On the other hand[56], documented that the isolates which are positive by phenotypic confirmatory test should be reported as resistant to all cephalosporins (except the cephamycin, cefoxitin and cefotetan) and aztreonam, regardless minimum inhibitory concentration (MIC) of cephalosporin.

The prevalence and relative distribution of ESBLs vary depending on the facility, the level of care taken to control nosocomial BSI, and the geographic location and time[57]. In the present setting, the percentage of ESBL-producing Gram-negative bacteria among BSI pediatric patients was 19.6%. Our finding is comparable to[53] who reported 22.6% ESBL producers out of a total of 11,886 member of Enterobacteriaceae isolated; and to[26] who reported 15.8% ESBL prevalence in blood cultures. However[58], reported ESBL production in 36% of Enterobacteriaceae from inpatients in a hospital in Riyadh.

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%) [59]. In the Arabian Peninsula, reported ESBL detection rates range from 8.5-38.5% in data from the Kingdom of Saudi Arabia[25,26,58,60,61] and (31.7%) in Kuwait[62]; the highest level of 41% is from the United Arab Emirates[63]. ESBL detection among inpatients and outpatients in a maternity unit in the eastern region of Saudi Arabia was reported as 27.5% but only *K. pneumoniae* isolates were studied[64].

ESBLs are occurred in hospital acquired infections (nosocomial infections) due to unhygienic conditions of the hospital environment[65]. In our setting, all ESBL producers were obtained from inpatients. Similarly[53], reported that the majority of ESBL producers (87.7%) were isolated from inpatients. Thus, infections associated with ESBL producers still remain largely nosocomial in nature. On contrary, emerging data from parts of Europe, Asia and South America indicates that community acquired infections caused by ESBL producing strains is now endemic in many countries[66].

A predominance of either *K. pneumoniae* or *E. coli* has often been reported among the ESBL isolates identified in different geographical regions[25,67, 68].

The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and in different institutes in Saudi Arabia [50]. The predominant ESBL producer in the current study was *K. pneumoniae* accounting for 45.5% of all ESBL isolates identified. A higher rate of ESBL production (55%) in *K. pneumonia* was reported in Riyadh; while, a lower prevalence rates were detected in Abha[64] and Al-Khobar[25]. The discrepancy may be due to differences in the type and volume of antibiotics consumed and differences in the time of collection of isolates.

Regarding *E. coli* isolates, ESBL production was detected in 4.5% of all isolates understudy. *E.coli* percentage was ~ 9.6% in other Saudi study[61]; 11.7% in a Kuwaiti study[62] and 1.2% in an Italian study[69].

A very important finding was noticed in this study as ESBL started to appear among *C. meningosepticum*, *S. marcescens*, *P. aeruginosa*, *Salmonella* spp. and *C. freundii*; which may reflect the expansion of the genes coding for ESBLs production to other bacterial genera.

In terms of risk factors for the acquisition of ESBL-producing isolates in this study, age was shown to be one of these factors. ESBL producers were more frequent in neonates and in NICU. Similarly, Mehrgan *et al.*,[70] reported ESBL production was most frequent in patients at the lower extreme of age. Neonates in NICU tended to be more debilitated, more likely to need ventilatory assistance, and had greater exposure to antimicrobial agents.

Another risk factor, is the length of hospital stay. In this setting, ESBL-producers were detected more in patients stayed for more than one month. Kim *et al.*[71] reported a longer mean length of hospital stay after the onset of bacteraemia in the cases of ESBL producing *K. pneumoniae* than in the cases of non-producing. Furthermore, ESBL production was associated with severe adverse outcomes, including higher overall and infection-related mortality, increased length of stay, delay in appropriate therapy, discharge to chronic care, and higher costs[72].

Mortality rate among the studied BSI pediatrics infected with ESBL organisms was 36.36%; and all of them was from ICUs. An explanation given by[73] for the high mortality rate, is that BSI by ESBL-producers is usually associated with a delay in the institution of appropriate antimicrobial therapy, as empirically instituted antibiotics may be inactive.

## CONCLUSION

The study showed the prevalence of ESBL-producers in 19.6% of the total studied Gram-negative BSI isolates. Thus, it is crucial for clinicians to be familiar with the clinical significance of these enzymes and understand their prevalence among pediatric patients in whom BSI is suspected.

This study also emphasizes the need of clinical microbiology laboratories to continue surveillance of ESBL producers especially at high-risk areas in the hospital as ICUs. We recommend ESBL tests to run in parallel with the main body of susceptibility testing instead of subsequently. This will inform the decision to provide empirical therapy active against the most likely pathogens until susceptibility test results are obtained. Strict infection control is also recommended to prevent trafficking of these ESBL producers from the hospital into the community.

**Competing interests**

Authors have declared that no competing interests exist.

**Ethical approval**

Ethical Committee of the Madinah Maternity and Children's Hospital & the Scientific Committee of Taibah University approved the study.

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