

Original Research Article

Analysis of blood stream infections: Antimicrobial susceptibility and associated types of extended spectrum β -lactamases

Lorina I Badger-Emeka^{1*}, Zainab Yaseen Al-Jaziri², Naheed Kausar¹, Nora Ahmad Al-Muhainy², Edric Estrella³

¹Department of Biomedical Sciences, College of Medicine, ²College of Medicine, ³Department of Public health, College of Applied Medical Sciences, King Faisal University. Al-Ahsa. Kingdom of Saudi Arabia

*For correspondence: **Email:** lbadgeremeka@kfu.edu.sa, lorinaineta@gmail.com

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Abstract

Purpose: To investigate the antimicrobial susceptibility and extended spectrum β -lactamase (ESBL) production by clinical isolates of *Escherichia coli* (ESBL-EC) and *Klebsiella* species (ESBL-KP) associated with blood stream infections (BSIs).

Methods: Bacteria isolation and identification were carried out using basic bacteriological and biochemical techniques. Antimicrobial susceptibility test was performed according to the guidelines of the Clinical Laboratory Standard Institute (CLSI). Vitek 2 Compact automated system was used to confirm the identifications (ID) and antimicrobial susceptibility test (AST). The ESBL produced by *E. coli* and *K. pneumoniae* isolates were phenotypically characterised using Modified Double Disc Synergy Test (MDDST), as recommended by CLSI.

Results: All (100 %) isolates were sensitive to imipenem and meropenem, while susceptibility to other antibiotics varied. ESBL genotypes, viz, *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX} were encountered in the BSIs. For ESBL-EC, TEM, SHV and CTX producers accounted for 33, 16.67 and 58.3 %, respectively, while 75, 91.7 and 100 % of ESBL-KP were TEM, SHV and CTX producers, respectively. In ESBL-KP, 67 % coexisted with all three genotypes (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX}), while 8.3 % of ESBL-EC coexisted with the three encoding genes (CTX, SHV and TEM).

Conclusion: ESBL *E. coli* and *K. pneumoniae* associated with BSI have been identified as TEM, SHV and CXT producers, with more ESBL-KP coexisting with all three than ESBL-EC.

Keywords: β -Lactamases, Genes, *Klebsiella pneumoniae*, *Escherichia coli*

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INTRODUCTION

Treatable bacterial infections are not considered as leading causes of death in patients. However, clinicians are encountering difficulties in treating bacterial infections globally, due to the

emergence of multidrug-resistant bacteria (MDR), more so with bacteria associated with blood stream infections (BSIs). The frequency of isolation of BSI varies globally, and also within geographical regions [1]. However, cases of BSIs are under-reported, indicating that many of them

are not included in the World Health Organization (WHO) list of most reported causes of death [2]. The annual reported neonatal deaths from BSIs is put at about 2.9 million in low income countries [4].

There is also a global increase in the emergence of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* which constitutes a high risk in healthcare-associated infections, including blood stream infections. This is of high significance due the role of ESBL producers as leading factors in morbidity and mortality. However, there is a general dearth of information on ESBL-BSIs due to under-reporting of incidents of BSIs, and the fact that when results were available, they were associated with inconsistency [3]. In England, Wilson *et al* [4] reported increased cases of BSIs up to 2006, followed by a decline therefrom to 2008. Moreover, available data for Danish population covered up to 2008 [5], while in Finland [6], increases in cases of BSIs covered up to 2007. Overall, a European surveillance study [7] has suggested an increase in cases of BSIs up to 2008. These reports point to either under-reporting of cases within the indicated periods, or a complete control of BSIs in these regions. However, in Saudi Arabia, reports on the incidence of BSIs have spanned over a period of two decades from 1983 [8] to a more recent date [9].

These reports imply that continuous attention is being drawn to BSIs generally in various regions in the Kingdom of Saudi Arabia, more so with its strategic location associated with high antimicrobial resistance. Al-Otaibi *et al* [10] gave an update on the prevalence of Gram-negative bacilli associated with BSIs in hospitalized patients with malignancy at an oncology unit in Riyadh. The report may be considered scanty since it did not include infections of blood stream attributed to ESBL-producing *Enterobacteriaceae* (ESBL-E) [11].

These infections have limited options for treatment, since they exhibit MDR to aminoglycosides and fluoroquinolones, thereby creating limited therapeutic options for clinicians. Therefore, it is not unlikely that patients are managed with inappropriate empirical therapy that would subsequently lead to an increase in morbidity and mortality. Another study investigated the risk factors associated with mortality in adult patients with ESBL-BSIs, and reported a three-fold increase in mortality resulting from inadequately-treated patients, relative to adequately-treated group of patients [12]. Beta-lactam antibiotics are placed amongst

the safest and most frequently prescribed antibacterial drugs worldwide [13]. This has led to persistent bacterial exposure to β -lactams, and subsequent mutations in the TEM, SHV, CTX-M genes of *Escherichia coli* and *Klebsiella pneumoniae*, the main ESBL producers [14,15].

The ESBLs belong to the molecular class A [15]. They are able to hydrolyze penicillins as well as third-generation cephalosporins and monobactams. It has been reported that CTX-M is the most common genetic variant of ESBL initially encountered in *E. coli* [15]. The SHV-1 encoding gene is chromosomally generated within *Klebsiella pneumoniae* responsible for resistance to broad-spectrum penicillins (ampicillin, tigecycline and piperacillin) [13]. This gene was later reported to have been incorporated into plasmids, with subsequent spread to other *Enterobacteriaceae* [13]. The TEM-1 was first reported in *E. coli* isolates. However, *Klebsiella oxytoca* harboring plasmid-mediated encoding TEM-12 gene was first reported in England in 1982 [14].

The presence of ESBLs such as ampicillin-hydrolyzing TEM-1 and SHV-1 β -lactamases in *Escherichia coli* and *Klebsiella pneumoniae*, and in other members of the *Enterobacteriaceae* is of immense microbiological and clinical importance. Moreover, the fact that these β -lactamases can spread to other pathogenic bacteria is of major public health concern. The resultant effect on mortality is evident in septic shock and organ failure, with *K. pneumoniae* producing more severe effects than any other *Enterobacteriaceae* [16]. To tackle the global problem created by drug-resistant bacteria, there is need for regular and concise reporting of global and regional surveillance, with a view to aiding rational use of antibiotic therapy. The present investigation studied the bacterial isolates associated with blood stream infections, their antimicrobial susceptibilities, and ESBL production of clinical isolates of *Escherichia coli* and *Klebsiella* species associated with blood infections.

EXPERIMENTAL

Ethical consideration

Permission for this research was obtained from the Postgraduate and Research Committee of College of Medicine KFU (approval code 18-02-19 B).

Bacteria isolation and characterisation

The ESBL-producing *Escherichia coli* and *Klebsiella* spp bacteria isolates were used for this

investigation. They were samples responsible for blood stream infections in hospitalised patients, and were collected for routine diagnosis required for patients care. All isolates were given laboratory IDs as shown in Tables 4 and 5. Bacteria isolation and their characterization were done using basic bacteriological and biochemical techniques. In the microbiology laboratory at the College of Medicine, further identification and confirmation of the clinical isolates were carried out using VITEK 2 compact automated system (BioMerieux, Marcy L'Etoile, France). Gram negative (GN) and antimicrobial susceptibility test (AST) cards were used for ID and antimicrobial susceptibility testing, respectively, according to the manufacturers' guidelines. The following antibiotics were used for the susceptibility test: ampicillin (10 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), cefuroxime (30 µg), cephalotin (30 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), piperacillin/tazobactam (100/10µg), tobramycin (10 µg), meropenem (10 µg), and imipenem (10 µg).

Screening for ESBLs

The ESBL produced by each of the isolates resistant to ceftriaxone and cefotaxime was phenotypically characterised using the modified double disc synergy test [MDDST] as recommended by CLSI [17]. Fresh cultures of the isolates were plated out on Muller-Hilton agar and discs of amoxicillin-clavulanate (20/10µg) along with the four cephalosporins: 3GC-cefotaxime, ceftriaxone, cefpodoxime and 4GC-cefepime. The method described by Kaur *et al* [18] was used for separating the distances between the 3GC and 4GC discs, with amoxicillin clavulanate disc (20/10µg) placed at the centre of the plate. The results were interpreted as described by CSLI [19] and Kaur *et al* [18]. Resistance to single test against cefotaxime and ceftazidime alone, and separately in combination with clavulanate were used as confirmatory tests. An increase in inhibition zone in the amoxicillin-clavulanate disc was interpreted as positive for ESBLs production. The positive and negative control strains for ESBLs production were ATCC *E. coli* 25922 and ATCC *K. pneumoniae* 70060, respectively. Only the ESBL producers were used for the investigation, out the 48 collected blood isolates.

Molecular analysis

Extraction and amplification of DNA were carried out using QIAgen DNA extraction kit (Qiagen, Germany) according to the manufacturer's

guidelines, using fresh cultures of overnight-grown ESBL-EC and ESBL-KP BSIs samples. The PCR amplification of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes was carried out for each of the isolates, while the presence of encoding ESBL gene of the resulting PCR products was visualised with agarose gel electrophoresis stained with ethidium bromide, using the primers shown in Table 1 [20-22]. A complete linkage cluster analysis was performed using Stata MP Version 13.

Statistical analysis

Data are presented as frequencies and proportions. An online MEDCALC statistical software was used for comparison of proportions. Values of $p < 0.05$ were considered statistically significant. GraphPad statistical software and STATA 12 SE- 64 software were used for cluster analysis.

RESULTS

Antimicrobial susceptibility

The results of antimicrobial resistance of *E. coli* and *K. pneumoniae* ESBLs isolates against antibiotic groups are shown in Figure 1. All (100 %) bacteria isolates investigated were resistant to the cephalosporins and monobactams. All *K. pneumoniae* BSIs were resistant to the penicillins, and also showed high resistance (72 %) to aminoglycoside. There was an observed variability in their antimicrobial sensitivities, with two-tailed test showing that the difference in antimicrobial resistance was not quite statistically significant between ESBL-EC and ESBL-KP. For aminoglycosides, although mean resistance of 42 and 72 % were observed for ESBL-EC and ESBL-KP, respectively, this difference was also not statistically significant. Similarly, for the fluoroquinolones, resistance values of 92 and 75 % were obtained for ESBL *E. coli* and ESBL *K. pneumoniae* respectively. Mean resistance of ESBL-EC to the penicillins was high (86 %), while all the ESBL-KP were intrinsically resistant to this group of antibiotics. However, all isolates were sensitive to the carbapenems. The results in Table 2 show that the BSIs were from male (50 %) and female (50 %) hospital wards, and ICU (42 %). The ages of the patients ranged from 8 months to 76 years.

Molecular profile

The genotypes TEM, SHV and CTX were found to be associated with ESBL-producing *E. coli* and *K. pneumoniae* BSIs, either alone or sometimes in coexistence of two or three of them. The CTX

gene was the most predominant in all (100 %) of the ESBL-KP isolates, while SHV and TEM were in 97.67 and 75 % of the isolates, respectively (Table 3). For the ESBL-EC, the CTX gene was predominant in 67 % of the isolates, while 33.33 and 17 % of the isolates carried SHV and TEM genes, respectively. The difference between the number of ESBL phenotypes in ESBL-EC and ESBL-KP was statistically significant ($p < 0.0001$; Table 3).

As regards the number of ESBL-producing genes encountered in the *E. coli* isolates, coexistence of all three i.e. bla_{TEM}, bla_{SHV} and bla_{CTX} was observed in 8.3 % of ESBL-EC, with an associated antimicrobial resistance of 76.47 % (Table 4). Isolates E5 and E11 with recorded resistance of 82.35 and 76.47 %, respectively, carried none of the three genes. Moreover, *E. coli* (E1) with a resistance of 82.35 % had only CTX associated with it. A large number of *K. pneumoniae* isolates (67%) coexisted with all three genes (bla_{TEM}, bla_{SHV} and bla_{CTX}), and resistance ranged between 76.47 - 88.24 % among these ESBL-KP BSIs. The remaining 33

% of ESBLs *K. pneumoniae* coexisted with either TEM or SHV, together with CTX, and the observed antimicrobial resistance ranged between 64.71 - 76.47 % (Table 5).

Results of analysis of the phylogenetic relatedness of ESBL-EC and ESBL-KP is shown in the dendrogram cluster (Figure 2). This analysis places all the isolates into 5 clades signifying dissimilarities between them, all based on resistance to the β -lactams against which they had been tested. Clade 1 consisted of E11, E9, E2, K9, K1, K7, and KO1, and showed similar susceptibilities to six different antibacterial agents namely amikacin, amoxicillin plus clavulanic acid, ciprofloxacin, gentamicin, tazocin and tobramycin. Clade 2 (E8 and E7) had similar susceptibilities to amikacin, amoxicillin plus clavulanic acid and tazocin. Results shown in Figure 2 placed the remaining isolates into different clades, all of which were based on their susceptibilities to antimicrobials against which they had been tested.

Table 1: Nucleotide sequence of primers used for the study

Primer	Nucleotide sequence (5' - 3')	Reference
TEM-F	AGATCAGTTGGGTGCACGAG	Yazdi et al [22]
TEM-R	CAGTGCTGCAATGATACCG	Yazdi et al [22]
CTX-M-U1	ATGTGCAGYACCAGTAARGTKATGGC	Mulvey et al [23]
CTX-M-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG	Mulvey et al [23]
SHV-F	GGGTTATTCTTATTTGTGCGC	Sima et al [24]
SHV-R	TTAGCGTTGCCAGTGCTC	Sima et al [24]

Table 2: ESBL-producing blood isolates from patients, ward of isolation and antimicrobial resistance

ESBL-producing <i>Escherichia coli</i>					ESBL-producing <i>Klebsiella pneumoniae</i>				
Lab ID	Gender	Age (years)	Ward	Resistance (%)	Lab ID	Gender	Age (years)	Ward	Resistance (%)
EC 1	F	50	Ward	82.35	KP 1	F	8Months	Ward	76.47
EC 2	M	60	ICU	76.47	KP 2	F	34	ICU	76.47
EC 3	M	14	Ward	58.82	KP 3	M	8Months	Ward	76.47
EC 4	F	29	ICU	82.35	KP 4	M	60	Ward	88.24
EC 5	M	76	Ward	58.82	KP 5	M	30	ICU	70.59
EC 6	F	76	Ward	70.59	KP 6	M	30	Ward	64.71
EC 7	M	68	Ward	76.47	KP 7	M	72	ICU	82.35
EC 8	M	68	Ward	58.82	KP 8	M	77	ICU	83.35
EC 9	M	35	ICU	76.47	KP 9	M	77	ICU	76.47
EC 10	M	43	ICU	70.59	KP 10	M	33	Ward	82.35
EC 11	M	35	Ward	76.47	KP 11	M	23	Ward	83.35
EC 12	M	22	Ward	70.59	KP 12	M	74	ICU	76.47
		months							82.35

Table 3: Genotypes encountered amongst ESBL-producers

Genotype	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	P-value
blaTEM	8(33.33%)	18(75%)	0.0001*
blaSHV	4(16.67%)	22(91.67%)	0.0001*
blaCTX	16 (67%)	24(100%)	0.0001*

*Chi-squared significance level $p < 0.0001$; TEM, SHV and CTX were statistically significant for *E. coli* and *K. pneumoniae* genes, respectively ($p = 0.000$). However, CTX did not show any statistical difference with either *E. coli* or *K. pneumoniae*.

Table 4: Phenotypic characteristics of ESBL-producing *Escherichia coli* blood isolates, their antimicrobial resistance, and associated genes

Lab ID	Ward of Isolation	Types of β -lactamases	Antimicrobial resistance pattern
EC 1	ward	CTX	AMK, AM, AUG, AZT, CAZ, PIME, CXM, CAX, CF, CIP,
EC 2	ICU	CTX	AM, AUG, AZT, CAZ, PIME, CXM, CAX, CTX, CF, CIP, GM, PI, FO.
EC 3	ward	CTX	AM, AUG, AZT, CAZ, PIME, CXM, CAZ, CTX, CF, PI
EC 4	ICU	CTX	AM, AZT, CAZ, PIME, CXM, CAZ, CTX, CF, CIP, PI
EC 5	Ward	NF	AMK, AM, AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, MER, PI, TO, LVF.
EC 6	Ward	SHV	AM, AZT, CAZ, PIME, CXM, CAX, CTX, CF, CIP, PI, LVF
EC 7	Ward	CTX, SHV	AM, AZT, CAZ, PIME, CXM, CAX, CTX, CF, CIP, GM, PI, TO, LVF.
EC 8	Ward	CTX, SHV	AM, AZT, CAZ, PIME, CXM, CAX, CTX, CF, CIP, GM, PI, LVF.
EC 9	ICU	CTX, SHV, TEM	AMK, AM, AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PI, TO, LVF.
EC 10	ICU	CTX	AMK, AM, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, PI, LVF.
EC 11	Ward	NF	AMK, AM, AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PI,
EC 12	Ward	TEM	AMK, AM, AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, PTZ, PI, TO, LVF.

AMK = amikacin, AM = ampicillin, AUG = amoxicillin/clavulanic acid, AZT = aztreonam, CAZ = ceftazidime, PIME = cefepime, CMX = cefuroxime, CAX = ceftriaxone, CTX = cefotaxime, CF = cephalothin, CIP = ciprofloxacin, GM = gentamicin, MER = meropenem, PTZ = piperacillin/tazobactam, TO = Tobramycin, LVF = levofloxacin, PI = piperacillin. NF = not found

Table 5: Phenotypic characteristics of ESBL-producing *K. pneumoniae* blood isolates, their antimicrobial resistance and the associated genes

Lab ID	Isolation ward	Type of β -lactamase	Antimicrobial resistance pattern
KP 1	Ward	CTX, SHV	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PT, TO, LVF.
KP 2	ICU	CTX, SHV,	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PT, TO, LVF.
KP 3	Ward	CTX, SHV, TEM	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PTZ, PT, TO, LVF.
KP 4	Ward	CTX, SHV, TEM	AMK, AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PTZ, PT, TO, LVF.
KP 5	ICU	CTX, SHV, TEM	AMK, AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PTZ, PT, TO, LVF.
KP 6	Ward	CTX, SHV	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PT, TO, LVF.
KP 7	ICU	CTX, TEM	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PTZ, PT, TO, LVF.
KP 8	ICU	CTX, SHV, TEM	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PTZ, PT, TO, LVF.
KP 9	ICU	CTX, SHV, TEM	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PT, TO, LVF.
KP 10	Ward	CTX, SHV, TEM	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PT, TO, LVF.
KP 11	Ward	CTX, SHV, TEM	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PT, TO, LVF.
KP 12	ICU	CTX, SHV, TEM	AUG, AZT, CAZ, PIME, CMX, CAX, CTX, CF, CIP, GM, PTZ, PT, TO, LVF.

AMK = amikacin; AUG = amoxicillin/clavulanic acid, AZT = aztreonam, CAZ = ceftazidime, PIME = cefepime, CMX = cefuroxime, CAX = ceftriaxone, CTX = cefotaxime; CF = cephalothin; CIP = ciprofloxacin; GM = gentamicin; MER = meropenem; PTZ = piperacillin/tazobactam; TO = tobramycin; LVF = tevfloxacin; PI = piperacillin

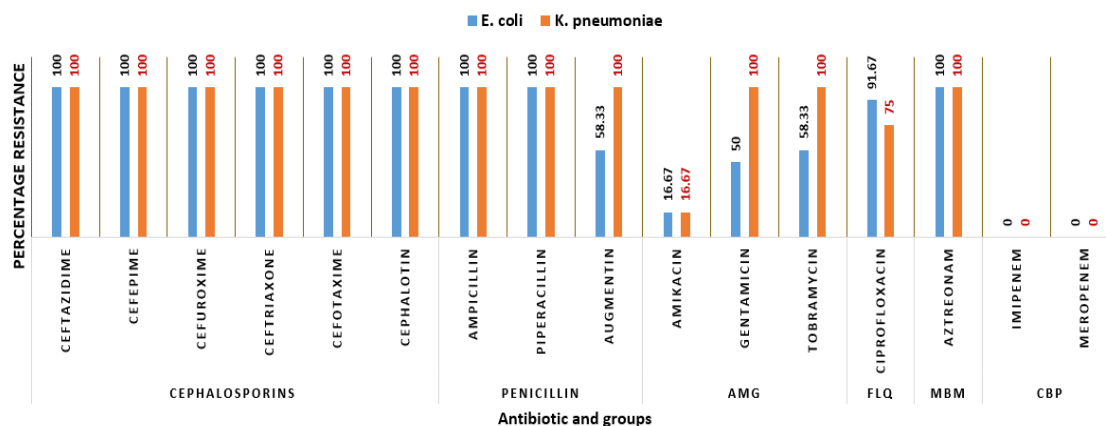


Figure 1: Antimicrobial resistance of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* blood isolates against antibiotics groups. AMG = aminoglycosides, FLQ = fluoroquinolones, MBM = monobactams, CBP = carbapenems. With two-tailed test, $p = 0.0580$ (differences not statistically significant)

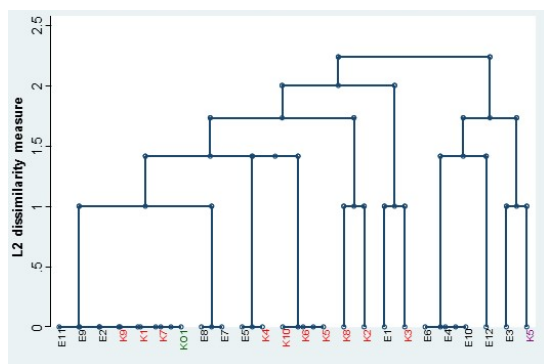


Figure 2: Dendrogram of cluster analysis of ESBL-producing blood isolates. **Key:** E = *Escherichia coli*; K = *Klebsiella pneumoniae*

DISCUSSION

This investigation has further highlighted the threat faced by clinicians in the treatment of patients in healthcare settings as a result of ESBL-KP and EBLs-EC BSIs due to the extensively high levels of antimicrobial resistance. High resistance such as was seen in the ESBL-KP and EBLs-EC BSIs in the present investigation indicates that there could be limited treatment options available to clinicians in healthcare settings [11]. Reports by the infectious Disease Society of America [25] listed ESBL-KP and ESBL-EC among the six MDR bacteria to which there is a need for urgent treatment options.

More than a decade later, that report remains valid, as is evident in the results of the present study with respect to antimicrobial susceptibility pattern exhibited by the ESBL-KP and EBLs-EC associated with BSIs. This implies that available therapeutic measures are grossly limited, and

this could lead to an increase in treatment failures and an increase in death rates [9,12]. In a study of BSIs caused by ESBL-E in a tertiary hospital in New Zealand, Freeman *et al* [11] reported higher mortality in ESBL-E bacteremia than in the control cases, with one of the risk factors being exposure to antibiotic treatment. Although the present report did not include treatment outcomes, there will be a need for continuous and more detailed updates on ESBL-producing BSIs, for a much needed global and regional surveillance.

In terms of molecular analysis, the present findings show that cefotaximase (CTX-M), temoneira (TEM) and sulfhydryl variable (SHV) were associated with ESBL-KP and EBLs-EC BSIs. There were variabilities in percentages of TEM, SHV and CTX encountered by the ESBL-KP and EBLs-EC associated with BSIs in this study, with the predominant gene being CTX. This is similar to the findings of Shahid *et al* [26]. Moreover, Scheuerman *et al* [24] reported that CTX-M was the most prevalent ESBL subtype identified in their study, while among the four classes of ESBLs, the A class TEM and SHV variants were the most-produced by *Klebsiella* species and *E. coli*. This suggests variations that could be attributed to regional differences [26]. Attention has been drawn to a global rise in the CTX-M-type ESBLs in community-acquired *E. coli*, as well as in nosocomial *Klebsiella* species [23], a view supported by other researchers [22]. Therefore, the present finding regarding 67 and 100 % CTX expressed by EBLs-EC and ESBL-KP, respectively, in the BSIs are similar to those reported previously [22].

Resistance by ESBL-producing bacteria responsible for these BSIs can be attributed to the number of β -lactamase genes encountered in

each bacterium, as shown in the results. The carriage of SHV β -lactamase by 92 % of ESBL-KP BSIs as seen in this investigation, as well as all isolates being CTX- β -lactamase producers, would explain the higher resistance against antimicrobials shown by them, relative to those of ESBL-EC BSIs. The high percentage carriage of the associated genes in the ESBL-KP BSIs in this investigation could be attributed to both chromosomal and extra-chromosomal factors. Similar views have been expressed in a previous study [26]. There were variations in the number of carriages of A class bla_{TEM}, bla_{SHV}, and bla_{CTX} genotypes in each of the BSIs in this study. This contributed to the level of resistance by each of the ESBL-KP and ESBL-EC isolates in the present investigation.

In addition, the coexistence of different β -lactamase genes within an isolate for both bacterial species could point to a mechanism that may lead to resistance against different antimicrobials as previously expressed [22]. However, observations on co-existence of antimicrobial resistance genes vary. Sima *et al* [22] found that ESBL-producing *E. coli* from urine sample isolates had two or more ESBLs genes, with the most common being the CTX and TEM genotypes. Similarly, other findings [27] found TEM and CTX to be the most common genotypes associated with *E. coli* in Saudi Arabia. In this investigation, the CTX and TEM genotypes were also the most common among ESBL-EC BSIs, while the most common for ESBL-KP were the SHV and CTX genotypes. These findings highlight differences in resistance genes among the bacterial species, as earlier reported [23]. With only 25 % of the isolates carrying multiple ESBL genes, there are possibilities of regional and geographical differences. Differences in gene carriage could also be due the types of specimens, as well as their origins (nosocomial or community). While the samples used by some researchers were from urine specimens [23,25], the samples used in this study were from blood stream infections. Another contributory factor associated with multiple carriage of resistant genes could be the length of hospitalisation of patients in intensive care unit (ICU), which was more than 14 days [25].

Therefore, there is the possibility that the ESBL-KP in the present investigation were nosocomial in origin. For treatment, the carbapenems remain the antibiotics of choice, since all ESBL producers in this investigation were sensitive to them. Moreover, another study [23] reported 8 % resistance to the carbapenems, indicating that the carbapenems remain the antimicrobial of

choice. On the other hand, with the dendrogram clustering results showing that both ESBL-EC and ESBL-KP BSIs were not of the same homologous group, there is a need to take this point into consideration when instituting treatment by healthcare providers, as had been suggested by previous other researchers [25]. In accordance with global recommendations for the monitoring and the reporting of antimicrobial resistance, this study serves as a guide for preventive measures aimed at mitigating BSIs in hospitals in the Kingdom of Saudi Arabia.

CONCLUSION

This study has revealed that bla_{TEM}, bla_{SHV} and bla_{CTX} phenotypes are associated with ESBL-producing BSI resulting from *K. pneumoniae* and *E. coli*. All three genotypes were predominant in ESBL-KP than ESBL-EC. With all BSI isolates being sensitive to the carbapenems, this group of antibiotics could be the gold standard for the treatment of *K. pneumoniae* and *E. coli* infections.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Lorina I. Badger-Emeka developed the research concept and study design, participated in all laboratory experiments, and in collection and analysis of data, drafting of report, and final report preparation. Zainab Y. Al-Jaziri participated in collection of laboratory specimens, and was involved in molecular DNA extraction, and in final preparation of report. Naheed Kausar took part in bacteriological experiments, amplification of DNA products and final preparation of the report. Nora Ahmad Al-Muhainy was involved in collection of laboratory specimens, molecular DNA extraction, and preparation of the report. Edric Estrella

carried out data analysis and final report preparation.

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