Emergence of bla_{TEM}, bla_{CTX-M}, bla_{SHV} and bla_{OXA} genes in multidrug-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* in Saudi Arabia

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Abstract. Multidrug-resistant (MDR) patterns due to extended-spectrum β-lactamase (ESBL) production in pathogenic bacteria are now becoming prevalent in hospitals worldwide, posing a public health challenge. The aim of the present study was to determine the antibiotic susceptibility patterns and distribution of the bla_{TEM}, bla_{CTX-M}, bla_{SHV} and bla_{OXA} ESBL resistance genes in MDR *Enterobacteriaceae* and *Acinetobacter baumannii (A. baumannii)*. A cross-sectional study was conducted between September 2017 and August 2018 in the King Abdullah Hospital (Bisha, Saudi Arabia). Bacterial isolates were collected from the clinical samples of patients; these were identified and screened for ESBL production and their antibiotic susceptibility was examined using standard microbiology methods. Multiplex-PCR runs were performed to identify genes encoding ESBL producers. DNA sequencing analysis was used to identify the specific gene variants. Of the 274 isolates, 173 (63.1%) exhibited MDR patterns to different antibiotics. *A. baumannii* revealed the highest resistance rates for cefuroxime (100%), gentamicin (88%) and amikacin (86%). *Klebsiella pneumoniae (K. pneumoniae)* isolates had the highest resistance rates for cefuroxime (98%), aztreonam and trimethoprim/sulfamethoxazole (87% for each). *Escherichia coli (E. coli)* exhibited high resistance rates for trimethoprim/sulfamethoxazole (92%) and cefuroxime (87%). Of the 173 MDR isolates, 78 (45.1%) exhibited ESBL production. Of these, 88.9% (72/78) carried ESBL genes. The most prevalent gene-encoding isolates were bla_{TEM} (84.7%), followed by bla_{CTX-M} (33.3%), bla_{SHV} (2.7%) and bla_{OXA-1} (1.4%). A single bla_{TEM} gene was predominantly produced by *K. pneumoniae* (60.7%), *A. baumanni*i (78.9%) and *Proteus mirabilis* (80%), whereas bla_{CTX-M} was harbored by *E. coli* (33.3%). The co-existence of two different genes in a single bacterium was revealed in 22.2% of isolates, commonly between bla_{TEM} and bla_{CTX-M} (19.4%). Sequencing analysis revealed that $bla_{CTX-M-15}$ and bla_{TEM-1} were predominant variants of the bla_{CTX-M} and bla_{TEM} genes, respectively. The present study revealed a diversity of ESBL genes in Gram-negative bacterial isolates, with bla_{TEM} being the most prevalent type. The emergence of various ESBL genes with several co-existing genotypes is alarming, rendering extensive surveillance studies necessary to understand the transmission and epidemiology of such resistant gene-carrying isolates.

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Introduction

In recent years, the production of extended-spectrum β-lactamases (ESBL) has become the main mechanism of resistance to β-lactam and other antibiotics in *Enterobacteriaceae* and *Acinetobacter baumannii (A. baumannii)* (1). ESBL enzymes confer resistance to penicillins, cephalosporins, monobactams and other antibiotic classes (2). Multidrug-resistant (MDR) patterns due to ESBL production in pathogenic bacteria are now becoming prevalent in hospitals worldwide, posing a public health challenge, including treatment failure, prolonged hospital stay and increased mortality rates (3-5).

Recently, >300 different ESBL types have been described in Gram-negative bacteria (6,7). The bla_{TEM} and bla_{SHV} types have been recognized as the most prevalent ESBL genes conferring antibiotic resistance in pathogenic bacteria worldwide (8-10). Previous studies have revealed that the number of clinical isolates harboring the bla_{CTX-M} gene type has also increased in the last few years (2,11). The bla_{CTX-M} family includes >130 β-lactamase variants classified into five distinct groups: $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-8}$, $bla_{CTX-M-9}$ and $bla_{CTX-M-25}$ (12).

The genetic diversity of ESBL-producing *Enterobacteriaceae* and *A. baumannii* has progressively increased, posing challenges to hospital authorities due to their ability to confer antibiotic susceptibility and limit therapeutic options (13,14). The characterization of resistance genes encoding ESBL-producing microorganisms is a powerful tool for developing evidence-based guidelines for combating antibiotic resistance in the clinical setting (11).

In Saudi Arabia, Gram-negative bacteria-harboring ESBL resistance genes have been studied, with most studies emerging from the central and eastern regions (5,15-19). Despite the spread of antibiotic resistance among bacterial pathogens in the southern region, data on the distribution of ESBL resistance genes and their resistance profile among *Enterobacteriaceae* and *A. baumannii* remain limited (20,21). A previous study performed at the Aseer Central Hospital, a regional hospital in the southern region, identified MDR patterns among 98.1% of *Acinetobacter* species recovered from patients at intensive care units (22). In addition, another study conducted in the same hospital identified a high distribution of class D carbapenemase-encoding genes in *A. baumannii*, mainly ISAba1/OXA-23 and ISAba1/OXA-24 carbapenemases, which is alarming and presents an emerging public health threat (23). The emergence of MDR *A. baumannii* bacteremia in the southern region of Saudi Arabia has been well documented as an important health problem (22,24,25). A recent study reported the high frequency of MDR Gram-negative bacteria and a rate of ESBL production of 27% in patients at the King Abdullah Hospital, a referral hospital in Bisha, in the southern region of the country (20).

Due to the lack of information on the genotyping of ESBL producers and their MDR patterns in southern Saudi Arabia, the present study aimed to determine the antibiotic susceptibility patterns and distribution of ESBL genes in *Enterobacteriaceae* and *A. baumannii* isolates collected from clinical specimens of patients. The findings of the present study facilitated the implementation of infection control measures and provided epidemiological data to prevent spreading of MDR bacteria. The data also provided guidelines for the use of antibiotics in the clinical settings and improved the management of patients suffering from infections caused by *Enterobacteriaceae* and *A. baumannii*.

Materials and methods

Study design and setting. A cross-sectional study was conducted between September 2017 and August 2018 at the King Abdullah Hospital (Bisha, Saudi Arabia). A total of 274 *Enterobacteriaceae* and *A. baumannii* were recovered from patients as a part of the treatment and diagnosis procedure for infections. The clinical samples were collected from the patients as a part of routine investigations of infectious agents in the hospital microbiology laboratory. Therefore, consent letters were not obtained from the patients as per the study nature. Various clinical samples of urine (n=96), sputum (n=84), wound swab (n=51), blood (n=27), high vaginal swab (n=5), tracheal aspirate (n=5), umbilical discharge (n=3), cerebrospinal fluid (n=2) and eye swab (n=1) collected from 274 patients suffering from different bacterial infections were included in the study. Discharge from umbilical stump was collected from neonatal and/or infant patients in the wards using sterile swab soaked with normal saline and submitted to the laboratory. High vaginal swab was taken from female patients by clinicians and sent for microbiological examinations. Clinical specimens with incomplete personal information of the patients were excluded from the study. The average age of the patients was 46.0±25.5 years, including 158 females and 116 males. In total, 32 patients were aged 2-17 years, 97 were aged 18-40 years, 57 were aged 41-65 years and 88 were aged >65years. Patients of <2 years old were excluded from the study. The hospital is a referral hospital in the southern region (365 beds) with different specialties serving the Bisha province and the surrounding areas. The Research and Ethics committee at the College of Medicine, University of Bisha (Bisha, Saudi Arabia) reviewed and approved the present study protocol (approval no. UBCOM/1438-05/04).

Isolation and identification of bacteria. *Enterobacteriaceae* and *A. baumannii* were collected from the microbiology laboratory of King Abdullah Hospital during the routine processing of the clinical specimens of patients. Preliminary isolation and identification of bacteria were based on conventional microbiological methods. Briefly, Isolation of bacterial pathogens from specimens of urine, stool, sputum and other body fluids were performed by inoculating one loopful of each sample onto MacConkey agar plates (Oxoid, Ltd.) using a sterile disposable plastic loop (10-μl loop). Specimens of wounds, eye, umbilical and vaginal swabs were inoculated directly onto MacConkey agar plates by streaking them onto a small area of the plate. A disposable sterile plastic loop (1-μl loop) was used for cross-streaking to spread the inoculum over the surface of each plate to obtain single colonies of the suspected bacterial pathogen. Specimen of blood (5 ml) were extracted under aseptic conditions, transferred immediately into sterile bottles containing brain heart infusion broth (Oxoid, Ltd.), incubated at 37˚C with 5% CO_2 and examine daily for turbidity for ≤7 days. If turbidity was observed, a 10-μl loopful of the blood sample was subcultured onto MacConkey agar plates for isolation of the suspected Gram-negative pathogen after aerobic incubation at 37˚C for 24 h. The isolate was tentatively identified based on the colony morphology, gram staining and oxidase test and the API 20 E Gram-Negative Microbial Identification Kit (cat. no. 20160; bioMerieux SA). Then, full identification of bacterial isolates was confirmed using the Phoenix system identification assay (Becton, Dickinson and Company). One single bacterial isolate from the clinical sample of each patient was included in the present study.

Screening of ESBL production. Phenotypic ESBL production among isolates was examined using a double-disc synergy test (DDST) as previously described (26) and the decreased susceptibility to cefuroxime, ceftazidime and cefotaxime was examined according to the Clinical and Laboratory Standard Institute (CLSI) recommendations (27). Bacterial isolates yielded positive results with DDST, when subjected to a multiplex-PCR amplification assay to detect bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$ and $bla_{\text{OXA-1}}$ resistance genes.

Antibiotic susceptibility testing of ESBL-producing bacteria. Antibiotic susceptibility testing of the *Enterobacteriaceae*

Table I. The frequency of extended-spectrum β-lactamase resistance genes among Gram-negative bacteria (n=72) as detected by multiplex PCR using different primers.

		Oligonucleotide primer (28)		
Resistance gene	N (%)	Sequence	Length	Band size (base pairs)
bla_{TEM}	61 (84.7)	F: 5'-CATTTCCGTGTCGCCCTTATTC-3'	22	800
		R: 5'-CGTTCATCCATAGTTGCCTGAC-3'	22	
bla_{CTX-M}	24 (33.3)			
bla_{CTX-M} group 1	20 (27.2)	F: 5'-TTAGGAARTGTGCCGCTGYA-3'	20	688
		R: 5'-CGATATCGTTGGTGGTRCCAT-3'	21	
bla_{CTX-M} group 2	3 (4.2)	F: 5'-CGTTAACGGCACGATGAC-3'	18	404
		R: 5'-CGATATCGTTGGTGGTRCCAT-3'	21	
bla_{CTX-M} group 9	3 (4.2)	F: 5'-TCAAGCCTGCCGATCTGGT-3'	19	561
		R: 5'-TGATTCTCGCCGCTGAAG-3'	18	
bla_{CTX-M} group 8/25	0.0	F: 5'-AACRCRCAGACGCTCTAC-3'	18	326
		R: 5'-TCGAGCCGGAASGTGTYAT-3'	19	
bla_{SHV}	2 (2.7)	F: 5'-AGCCGCTTGAGCAAATTAAAC-3'	21	713
		R: 5'-ATCCCGCAGATAAATCACCAC-3'	21	
bla_{OXA-1}	1 (1.4)	F: 5'-GGCACCAGATTCAACTTTCAAG-3'	22	564
		R: 5'-GACCCCAAGTTTCCTGTAAGTG-3'	22	

F, forward; R, reverse.

and *A. baumannii* was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar medium (Oxoid) as per the CLSI guidelines (27). The following antibiotics with known concentrations recommended by the CLSI were examined: Amikacin (30 μg), amoxicillin/clavulanate (20/10 μg), aztreonam (30 μg), cefepime (30 μg), cefotaxime (30 μg), cefoxitin (30 μg), ceftazidime (30 μg), cefuroxime (30 μg), ciprofloxacin (5 μg), colistin (10 μg), gentamicin (10 μg), imipenem (10 μg), meropenem (10 μg), nitrofurantoin (50 μg), piperacillin (100 μg), piperacillin/tazobactam (100/10 μg), tobramycin (10 μg) and trimethoprim/sulfamethoxazole (23.75 μg/1.25 μg; Oxoid). *E. coli* American Type Culture Collection (ATCC) 25922 served as a control strain for antibiotic susceptibility examination. The final antibiotic susceptibility results of bacterial pathogens were interpreted using the 2017 CLSI breakpoints to categorize the isolates as susceptible or resistant. All intermediate results were considered resistant strains. 'A susceptible category indicates that the isolates of the patient respond to the usually achievable concentrations of that antibiotic when the dosage is recommended to treat that type of infection and bacterial species. Conversely, the resistant category indicates that the isolates of the patient are not inhibited by the usually achievable concentrations of that antibiotic with the dosages usually used with that drug' (27). Isolates were defined as MDR when they were resistant to at least three antibiotics from different classes.

Multiplex-PCR for the detection of ESBL genes. Multiplex-PCR runs were performed to identify the bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{OXA-1} genes. A pair of six specific oligonucleotide primers (Eurofins Scientific) were used in the PCR reaction, as previously described (28). DNA was extracted from ESBL-producing isolates using the boiling method as previously described (29). The amplification of ESBL genes was then carried out in a total reaction volume of 50 μl. Each reaction mixture contained 25 μl HotStarTaq Plus Master Mix (cat. no. 203643; Qiagen GmbH), 4 μl DNA template, a variable volume of a specific primer group (Table I) and 9 μl nuclease-free water. The Eppendorf Master cycler Gradient instrument (Eppendorf) was used for the amplification of target genes with the following optimal cycling conditions: Initial heat activation at 95˚C for 5 min, 35 cycles of denaturation at 94˚C for 45 sec, annealing at 53˚C for 45 sec, extension at 72˚C for 1 min and a final extension at 72˚C for 10 min. The amplification products were visualized under ultraviolet illumination at a wavelength of 312 nm, after running at 85 volts for 60 min on 2% agarose containing ethidium bromide (1 μg/ml). A 100-bp DNA ladder (cat. no. 239045; Qiagen GmbH) was used as a standard molecular weight to determine the size of PCR products. DNA from reference bla_{CTX-M}, bla_{TEM}, bla_{SHV} and bla_{OXA}-like-positive strains was used as a positive control.

DNA sequencing. Random PCR products of bla_{TEM-}, bla_{SHV-}, bla_{CTX-M-} and bla_{OXA-1}-positive samples were selected for DNA sequencing to identify the specific gene subtypes. A total of ~30 μl PCR products were sealed in sterile Eppendorf tubes and sent to Macrogen, Inc. for sequencing. PCR products were sequenced on an ABI PRISM® 3730XL Analyzer (96 capillary types) using the same primer sets (Table I). The results were obtained from the website of the company.

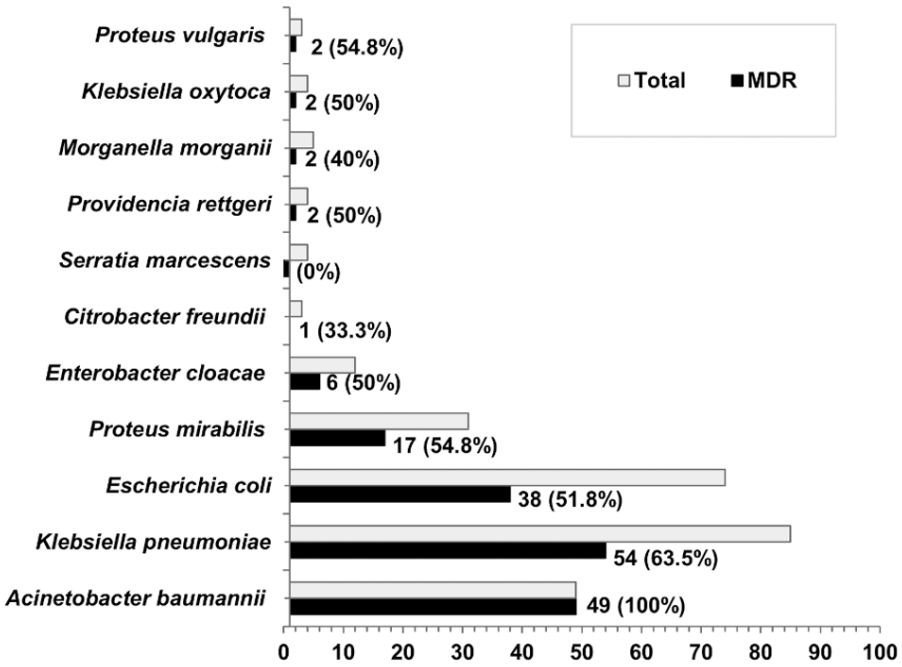


Figure 1. Frequency of multidrug-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* producing extended-spectrum β-lactamase. MDR, multidrug-resistant.

Similarities in the nucleotide sequences were compared on the GenBank database of the National Center for Biotechnology Information website using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences obtained for bla_{TEM}, bla_{CTX-M} and bla_{SHV} genes have been deposited in the GenBank database (http://getentry.ddbj.nig.ac.jp/) under accession numbers LC636038 to LC636063.

Statistical analysis. Statistical analysis was performed using SPSS 22.0 (IBM Corp.). Simple descriptive statistics were used to calculate antibiotic-resistant patterns, and the prevalence and distribution of ESBL resistance genes. Outcome data were stated as proportions in frequencies and means ± standard deviation.

Results

Bacterial isolates. A total of 274 *Enterobacteriaceae* and *A. baumannii* isolates obtained from the clinical samples of patients were used in the present study. The isolates were recovered from samples of urine, sputum, wound swab, blood, high vaginal swab, tracheal aspirate, umbilical discharge, eye swab and cerebral spinal fluid. This indicated that *Enterobacteriaceae* and *A. baumannii* could cause infections throughout body systems and sites, including genito-urinary, respiratory, bloodstream, central nervous and soft tissue infections, which was consistent with a recent study (30).

Resistance patterns of Enterobacteriaceae and A. baumannii. Out of the 274 isolates, 63.1% (n=173) exhibited MDR patterns to different classes of antibiotics. Fig. 1 illustrated the MDR patterns among different isolates. The MDR pattern was determined in 100% of *A. baumannii*, 63.5% of *Klebsiella pneumoniae (K. pneumoniae)*, 54.8% of *Proteus mirabilis (P. mirabilis)* and 51.8% of *E. coli* isolates.

Table II summarized the antibiotic susceptibility of MDR isolates. *A. baumannii* exhibited the highest resistance rates to the tested antibiotics (86% for amikacin, 88% for gentamicin and 100% for cefuroxime). *K. pneumoniae* exhibited the highest resistance rates for cefuroxime (98%), aztreonam and trimethoprim/sulfamethoxazole (87% each), and cefotaxime (83%). *E. coli* exhibited high resistance rates for trimethoprim/sulfamethoxazole (92%), cefuroxime (87%) and ceftazidime (71%). *P. mirabilis* exhibited high resistance rates for trimethoprim/sulfamethoxazole (100%), amoxicillin/clavulanate, cefotaxime, cefuroxime (88% each), cefepime, ciprofloxacin (82% each) and ofloxacin (77%).

Distribution of ESBL genes among MDR isolates. Of the 173 MDR *Enterobacteriaceae* and *A. baumannii* isolates, 78 (45.1%) exhibited ESBL production. Of the 78 isolates, 72 (92.3%) carried ESBL genes as determined using multiplex-PCR. Fig. 2 presents an example of multiplex-PCR results revealed during the present study. Of the 72 isolates, 53 were *Enterobacteriaceae* (*K. pneumoniae*, 28; *E. coli*, 18; *P. mirabilis*, 5 and *Enterobacter cloacae*, 2) and 19 were *A. baumannii* (Fig. 3). Collectively, the most prevalent ESBL resistance genes in the isolates were bla_{TEM} (84.7%) followed by bla_{CTX-M} (33.3%), bla_{SHV} (2.7%) and bla_{OXA-1} (1.4%). The most frequent bla_{CTX-M} group was the bla_{CTX-M}-group 1 (27.2%), followed by the bla_{CTX-M}-group 2 and 9 (4.2%), whereas none of the isolates carried the bla_{CTX-M}-group 8/25 (Table I).

Frequency of ESBL genes in Enterobacteriaceae and A. baumannii. Fig. 3 illustrates the distribution of ESBL genes

Table II. Percentage of antimicrobial resistance among multidrug-resistant *Acinetobacter baumannii* and *Enterobacteriaceae* family members.

Agent	*A. baumannii* (n=49)	*K. pneumoniae* (n=54)	*E. coli* (n=38)	*Proteus mirabilis* (n=17)	*Enterobacter cloacae* (n=6)	*Citrobacter freundii* (n=1)	*Providencia rettgeri* (n=2)	*Proteus vulgaris* (n=2)	*Morganella morganii* (n=2)	*K. oxytoca* (n=2)
Amikacin	86 (42)	41 (22)	16 (6)	35 (6)	33 (2)	0.0 (0)	50 (1)	50 (1)	50 (1)	50 (1)
Amoxicillin/ clavulanate	92 (45)	69 (37)	37 (14)	88 (15)	50 (3)	100 (1)	50 (1)	50 (1)	50 (1)	50 (1)
Aztreonam	96 (47)	87(47)	68 (26)	65 (11)	83 (5)	100 (1)	50 (1)	50 (1)	100 (2)	100 (2)
Cefepime	94 (46)	82 (44)	66 (25)	82 (14)	50 (3)	0.0 (0)	50 (1)	0.0 (0)	50 (1)	0.0 (0)
Cefotaxime	96 (47)	83 (45)	66 (25)	88 (15)	33 (2)	100 (1)	50 (1)	50 (1)	50 (1)	0.0 (0)
Ceftazidime	94 (46)	80 (43)	71 (27)	59 (10)	50 (3)	0.0 (0)	0.0 (0)	50 (1)	50 (1)	0.0 (0)
Cefuroxime	100 (49)	98 (53)	87 (33)	88 (15)	83 (5)	100 (1)	100 (2)	100 (2)	100 (2)	50 (1)
Ciprofloxacin	96 (47)	61 (33)	58 (22)	82 (14)	33 (2)	100 (1)	100 (2)	0.0 (0)	50 (1)	100 (2)
Colistin	6 (3)	4.0 (2)	3.0 (1)	6.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100 (2)	0.0 (0)
Foxitin	92 (45)	46 (25)	18 (7)	59 (10)	50 (3)	100 (1)	0.0 (0)	0.0 (0)	100 (2)	50 (1)
Gentamicin	88 (43)	61 (33)	37 (14)	53 (9)	17 (1)	0.0 (0)	50 (1)	50 (1)	100 (2)	0.0 (0)
Imipenem	94 (46)	35 (19)	13 (5)	29 (5)	17 (1)	0.0 (0)	50 (1)	50 (1)	100 (2)	0.0 (0)
Meropenem	92 (45)	33 (18)	13 (5)	24 (4)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100 (2)	50 (1)
Nitrofurantoin	96 (47)	69 (37)	37 (14)	71 (12)	67 (4)	0.0 (0)	100 (2)	0.0 (0)	50 (1)	50 (1)
Ofloxacin	94 (46)	63 (34)	50 (19)	77 (13)	33 (2)	100 (1)	100 (2)	0.0 (0)	50 (1)	100 (2)
Piperacillin	94 (46)	61 (33)	47 (18)	65 (11)	50 (3)	0.0 (0)	50 (1)	50 (1)	100 (2)	50 (1)
Piperacillin/ tazobactam	94 (46)	54 (29)	40 (15)	35 (6)	50 (3)	0.0 (0)	50 (1)	50 (1)	100 (2)	50 (1)
Tobramicin	90 (44)	59 (32)	37 (14)	53 (9)	33 (2)	0.0 (0)	50 (1)	50 (1)	50 (1)	50 (1)
Trimethoprim/ sulfamethoxazole	96 (47)	87 (47)	92 (35)	100 (17)	100 (6)	100 (1)	100 (2)	50 (1)	50 (1)	100 (2)

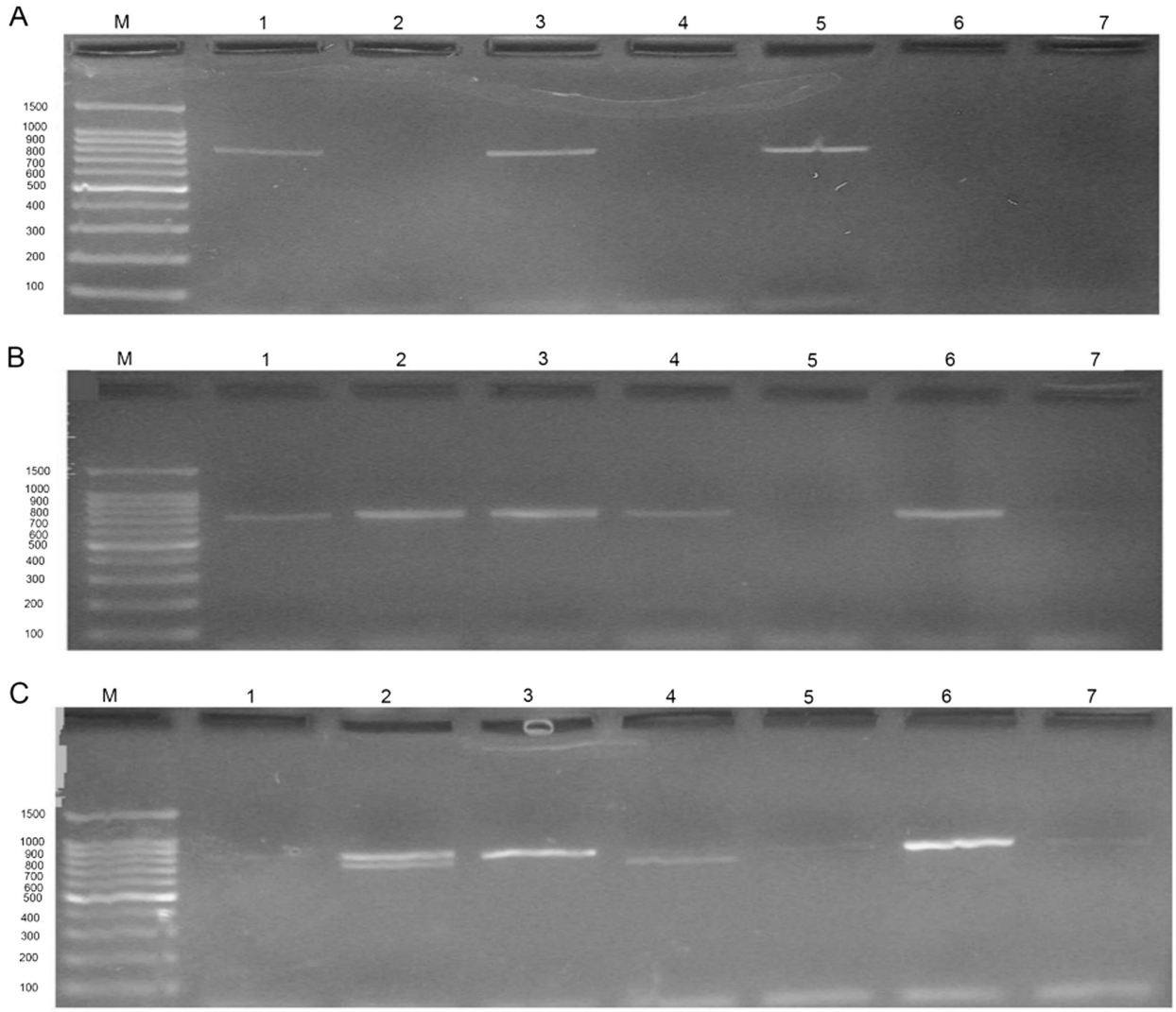


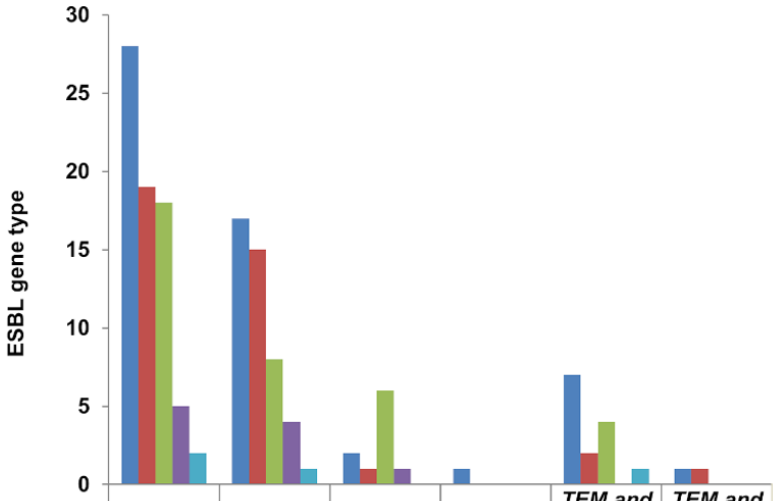
Figure 2. Agarose gel electrophoresis of multiplex-PCR amplification products. (A) *Acinetobacter baumannii* (lanes 1 and 3) and *Klebsiella pneumoniae* (lane 5) showed positive expression of the bla_{TEM} gene (800 bp). *Acinetobacter baumannii* (lanes 2, 4 and 6) and *Escherichia coli* (lane 7) showed negative findings to ESBL gene expression. (B) *Klebsiella pneumoniae* (lane 1), *Escherichia coli* (lanes 2, 3 and 6) and *Enterobacter cloacae* (lane 4), positive bla_{CTX-M} gene (688 bp). *Acinetobacter baumannii* (lane 5) and *Proteus mirabilis* (lane 7) showed negative findings to ESBL gene expression. (C) Lane 2, co-existence of bla_{TEM} and bla_{SHV} genes in *Klebsiella pneumoniae* isolate. *Acinetobacter baumannii* (lanes 3 and 6) showed positive bla_{TEM} gene expression. *Klebsiella pneumoniae* (lane 4) showed positive bla_{SHV} gene expression (713 bp). *Acinetobacter baumannii* (lanes 1, 5 and 7) showed negative findings to ESBL gene expression. Lane M, DNA marker (1,500-100 bp);. ESBL, extended-spectrum β-lactamase.

among Gram-negative bacterial isolates. A single bla_{TEM} gene was predominantly produced by *K. pneumoniae* (60.7%), *A. baumannii* (78.9%) and *P. mirabilis* (80%), while bla_{CTX-M} was commonly produced by *E. coli* (33.3%).

Out of the total PCR-positive isolates (n=72), the co-existence of two different genes in a single isolate was revealed in 16 (22.2%) strains. This combination was mainly observed between bla_{TEM} and bla_{CTX-M} (19.4%; 14/72) and between bla_{TEM} and bla_{SHV} (2.8%, 2/72) genes. Among the isolates carrying a combination of bla_{TEM} and bla_{CTX-M} genes (n=14), the majority were *K. pneumoniae* (50%, n=7) and *E. coli* (28.6%, n=4; Fig. 3).

Distribution of ESBL genes according to isolate sources. The distribution of various ESBL resistance genes among the clinical samples of patients is revealed in Table III. Bacterial isolates encoding various ESBL resistance genes were commonly recovered from sputum (n=30), followed by urine (n=18), wound (n=13) and blood (n=8) specimens. A single bla_{TEM} gene was frequently detected among isolates from sputum (73.3%; 22/30), urine (61.1%; 11/18) and wound (53.8%; 7/13) specimens. The highest frequency of a single bla_{CTX-M} gene was detected among wound isolates (23.1%; 3/13) compared with sputum (10%), blood (12.5%; 1/8) and urine (11.1%; 2/18) isolates. The co-existence of bla_{TEM} and bla_{CTX-M} genes was observed in 37.5% (3/8) of blood isolates, 23.1% (3/13) of wound isolates and 22.2% (4/18) of urine isolates.

Sequencing analysis of resistance genes encoding ESBL producers. The sequencing analysis of the bla_{CTX-M}, bla_{TEM} and bla_{SHV} types is revealed in Table IV. Out of the 20 bla_{CTX-M} family members, the majority (75%; n=15) carried the $bla_{CTX-M-15}$ subtype. $bla_{CTX-M-15}$ was identified among *K. pneumoniae* (n=7) and *E. coli* (n=5) isolates. Out of the 18 bla_{TEM} family members, TEM-1 was the most prevalent



	Total (n=72)	TEM (n=45)	CTX-M (n=10)	OXA-1 (n=1)	TEM and CTX-M (n=14)	TEM and SHV (n=2)
Klebsiella pneumoniae	28	17	2	1	7	1
Acinetobacter baumannii	19	15	1	0	2	1
Escherichia coli	18	8	6	0	4	0
Proteus mirabilis	5	4	1	0	0	0
Enterobacter cloacae	2	1	0	0	1	0

Figure 3. Prevalence and characterization of resistant genes encoding extended-spectrum β-lactamase-producing *Enterobacteriaceae* and *Acinetobacter baumannii*. ESBL, extended-spectrum β-lactamase.

variant (83.3%, n=15). This subtype was common among *A. baumannii* (n=7) and *K. pneumoniae* (n=5) isolates.

Discussion

The emergence of MDR patterns due to ESBL-producing *Enterobacteriaceae* and *A. baumannii* is becoming a global concern (13,31). The present study determined antibiotic susceptibility patterns and characterized ESBL genes among clinical isolates of *Enterobacteriaceae* and *A. baumannii*. Collectively, 63.1% of isolates exhibited MDR to different antibiotics. In addition, MDR patterns were identified in 63.5% of *K. pneumoniae* and 51.8% of *E. coli* isolates. In Riyadh, the capital of Saudi Arabia, MDR patterns were identified in 67% of uropathogenic *E. coli* isolates at a tertiary healthcare center (19). These values were higher than those reported in Libya, where the MDR phenotype was detected in 33.2% of *E. coli* and 42% of *K. pneumoniae* isolates from patients with urinary tract infections (32). The results were also consistent with the observed high prevalence of MDR patterns (100%) among clinical isolates of *Enterobacteriaceae* carrying ESBL resistance genes collected in Ethiopia (33).

In the present study, the overall resistance rates of MDR *Enterobacteriaceae* and *A. baumannii* were very high for most examined antibiotics, except for colistin. Previous studies have reported that co-resistance to several antibiotic classes of penicillins, cephalosporins, aminoglycosides, fluoroquinolones, trimethoprim/sulfamethoxazole and carbapenems was common among ESBL-producing Gram-negative bacteria (7,19,33,34). These findings indicated that the emergence of ESBL-producing microorganisms could cause susceptibility to various antibiotics.

A multiplex-PCR assay has been proposed to rapidly detect several resistance genes encoding ESBL-producing Gram-negative bacteria (28,35). However, complete gene sequencing for the bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{OXA-1} types is essential to differentiate narrow-spectrum β-lactamases from ESBL (35). In the present study, among 81 phenotypically identified ESBL isolates, 88.9% carried one or more of the following resistant genes: bla_{TEM}, bla_{CTX-M}, bla_{SHV} and bla_{OXA}. Previous studies have reported that bacteria carrying ESBL genes confer resistance to extended-spectrum cephalosporins, β-lactam agents and other antibiotic classes (7,19,20). This phenomenon may pose serious public health risks, as it would result in substantial limitations in therapeutic options. Thus, appropriate control measures, including establishing screening strategies for identifying ESBL-producing bacteria, are required to prevent such strains.

bla_{TEM} was the most prevalent gene detected in ESBL-producing Gram-negative bacteria in the present study. This was inconsistent with a study from the Eastern region of Saudi Arabia, where bla_{CTX-M} (97.4%) was more frequent than bla_{SHV} (23.1%) and bla_{TEM} (0.0%) in *Enterobacteriaceae* (16). Similarly, the predominance of the bla_{CTX-M} type in ESBL-producing Gram-negative bacteria in the Eastern region have been documented by other studies (4,36). Worldwide studies have reported different ESBL resistance genes produced by Gram-negative bacteria. For instance, bla_{CTX-M} was the most prevalent type in the Asian Pacific region, followed by bla_{SHV} and bla_{TEM} (1). In Nigeria, the most frequent gene types among isolates from patients with surgical site infections were bla_{SHV}, bla_{CTX-M} and bla_{OXA} (37). In Burkina Faso, the most prevalent ESBL resistance genes were bla_{CTX-M} (40.1%), bla_{TEM} (26.2%) and bla_{SHV} (5.9%) in *Enterobacteriaceae* (38).

Table III. Distribution of extended-spectrum β-lactamase resistance genes in Gram-negative isolates recovered from clinical samples of patients.

Source of Gram-negative bacteria	Single gene			Combined genes		Total
	bla_{TEM} (n=45)	bla_{CTX-M} (n=10)	bla_{OXA-1} (n=1)	bla_{TEM} and bla_{CTX-M} (n=14)	bla_{TEM} and bla_{SHV} (n=2)	
Sputum (n=30)	22	3	1	3	1	30
Urine (n=18)	11	2	0	4	0	18
Wound (n=13)	7	3	0	3	0	13
Blood (n=8)	4	1	0	3	0	8
Tracheal aspirate (n=2)	0	1	0	1	0	2
Eye swab (n=1)	1	0	0	0	0	1

Table IV. Sequencing analysis results of bla_{CTX-M}, bla_{TEM} and bla_{SHV} genes produced by *Enterobacteriaceae* members and *Acinetobacter baumannii*.

ESBL gene	Total (n=40)	*K. pneumoniae* (n=13)	*E. coli* (n=12)	*P. mirabilis* (n=2)	*E. cloacae* (n=3)	*A. baumannii* (n=10)
bla_{CTX-M} subtype (n=20)						
CTX-M-15	15	7	5	1	1	1
CTX-M-71	1	0	1	0	0	0
CTX-M-101	1	0	0	0	0	1
CTX-M-127	1	0	1	0	0	0
CTX-M-181	1	0	0	0	1	0
CTX-M-182	1	0	1	0	0	0
bla_{TEM} subtype (n=18)						
TEM-1	15	5	2	0	1	7
TEM-115	1	0	1	0	0	0
TEM-159	1	0	0	1	0	0
TEM-169	1	0	1	0	0	0
bla_{SHV} subtype (n=2)						
SHV-28	1	0	0	0	0	1
SHV-226	1	1	0	0	0	0

These results, coupled with the present findings, revealed that the prevalence of ESBL gene types can vary between locations and geographical regions.

MDR ESBL-producing *K. pneumoniae* and *A. baumannii* have become common causes of healthcare-related infections (11,31). In the present study, the prevalence of bla_{TEM} in *K. pneumoniae* was revealed to be 60.7%. Increasing rates of the bla_{TEM} gene have been reported among clinical isolates of *K. pneumoniae* in Al-Qassim (70.9%) (8) and Riyadh (54.05%), in the Central region of Saudi Arabia (17). On the other hand, the frequency of bla_{TEM} was high among *A. baumannii* isolates, which was consistent with a previous study from the Makkah city in the western region of the country (31). These high rates, which indicated the dissemination of such ESBL-producing isolates, is alarming for multiple hospitals. The high prevalence of the bla_{TEM} gene detected in *K. pneumoniae* and *A. baumannii* isolates may increase the incidence rate of infection caused by these ESBL producers across different regions. This renders extensive surveillance studies in local and national hospitals in Saudi Arabia necessary to understand the transmission and epidemiology of resistance genes encoding ESBL-producing bacteria. However, using molecular methods in local hospitals to detect resistance genes may help develop effective

new antimicrobial treatments against ESBL producers and improve the infection control system. In the present study, *E. coli* commonly produced the $bla_{\text{CTX-M}}$ gene (33.3%). Similarly, the predominance of *E. coli* carrying $bla_{\text{CTX-M}}$ genes has been reported in the western region of Saudi Arabia (39). Previous studies revealed that $bla_{\text{CTX-M}}$ is the most prevalent gene among uropathogenic *E. coli* isolates from patients with hospital and community-acquired infections (19,29). Furthermore, ESBL-producing *E. coli* collected from fecal colonization was revealed to produce the $bla_{\text{CTX-M}}$ gene as it has been reported in a previous study (40). Several factors and mechanisms contribute to the spread of bacterial clones carrying the $bla_{\text{CTX-M}}$ gene in Saudi Arabia, including plasmid dissemination and the clonal spread of bacterial strains, the frequent use of cephalosporins and the large population of migrant workers (13). According to Yasir *et al* (39), the high diversity in the *E. coli* clones may have arisen due to the fact that ~50% of the population of Saudi Arabia are expatriates from developing countries, including Pakistan, India, Bangladesh, the Philippines and African countries where self-medication in patients is evident.

The present study revealed that $bla_{\text{CTX-M-15}}$ was the most frequent subtype of the $bla_{\text{CTX-M}}$ type. This was consistent with data from Saudi Arabia (6,16,17) and several other parts of the world (1,2). These findings indicated that $bla_{\text{CTX-M-15}}$ is a public health concern, since it is the most widespread gene worldwide. The emergence of the $bla_{\text{CTX-M-15}}$ variant has been revealed to be attributed to the horizontal gene transfer of genetic elements and the clonal expansion of microorganisms (19,41). Furthermore, the widespread and unnecessary use of ceftriaxone and cefotaxime have contributed to the emergence and spread of $bla_{\text{CTX-M}}$ resistance genes (2).

Multiple ESBL resistance genes in a single bacterium render that strain more difficult to treat with several antibiotic drugs (16). In the present study, the co-existence of two different ESBL genes in the same strain was detected in 22.2% of isolates. However, the most common combination of ESBL resistance genes was between bla_{TEM} and $bla_{\text{CTX-M}}$, which was consistent with studies from Pakistan (42) and Algeria (12). However, the combined production of bla_{TEM} and $bla_{\text{CTX-M}}$ genes was more frequently detected in *K. pneumoniae* (50%) and *E. coli* (28.6%) isolates. These figures were lower than those reported in Nepal, where two or more ESBL genes were present in 100% of *Klebsiella spp.* and 56.2% of *E. coli* clinical isolates from a teaching hospital (7). The value reported in the present study was considerably higher than the 3.4% reported in uropathogenic *E. coli* from the Eastern region of Saudi Arabia (29).

The present findings revealed that sputum was the most frequent source of various ESBL resistance genes in Gram-negative bacteria. This may be due to the several sputum samples collected from patients at intensive care unit (ICU) wards. A previous study indicated that the characterization of antibiotic susceptibility of bacterial pathogens from the sputum of patients in the ICU with ventilator-associated pneumonia can help control this type of infection (43). In addition, it is known that most patients admitted to the ICU are immunocompromised and/or undergoing invasive procedures, which would lead to prolonged antibiotic therapy (25). The extended stay, selective pressure and frequent use of antibiotic treatment of patients in the ICU contribute to the increase in ESBL producers (43).

The present study has several limitations that need to be addressed in future studies. Firstly, the study was laboratory-based; therefore, clinical data of the patients were not obtained to analyze the risk factors for ESBL infection and understand the epidemiological spread of ESBL genes. Secondly, the study was conducted in a single center in southern Saudi Arabia. The results can therefore not be representative of all parts of the southern region. Multicenter studies are required to confirm these findings. Thirdly, the AmpC β-lactamase class and other types of ESBL enzymes, (such as bla_{VEB}, bla_{PER}, bla_{GES} and bla_{BEL}) which confer significant antibiotic resistance among Gram-negative bacteria, were not examined.

In conclusion, the ESBL resistance genes were a significant cause of MDR patterns and conferred susceptibility to various antibiotic agents in *Enterobacteriaceae* and *A. baumannii*. The present study reported high levels of various resistance genes in ESBL-producing isolates, with bla_{TEM} being the most prevalent type. In addition, the co-existence of two different ESBL genes has been frequently detected in a single bacterial pathogen (12,42). The $bla_{\text{CTX-M-15}}$ gene is the predominant variant among isolates carrying the $bla_{\text{CTX-M}}$ type. The emergence of various ESBL-resistant and coexisting genes in *Enterobacteriaceae* and *A. baumannii* is alarming and may significantly limit the efficacy of therapeutic options in hospital settings. However, extensive surveillance studies at both the local and national levels are urgently required to obtain an understanding of the transmission and epidemiology of resistance genes in ESBL-producing bacteria. Using molecular methods at local hospitals to detect resistance genes in ESBL-producing bacteria is recommended to improve the infection control system and help set effective antibiotic therapy plans.

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Availability of data and materials

All data generated or analyzed during the study are included in this published article. The datasets generated during the present study are available in the Genbank repository, (http://getentry.ddbj.nig.ac.jp/; accession numbers LC636038- LC636063).

Authors' contributions

MEI, TBA, MA and BKE conceived the idea of the study and developed the protocol. MEI and MA designed and conducted the study. MEI and TBA analyzed and interpreted the data and wrote the initial draft. MEI, TBA, MA, BKE reviewed the literature. BKE revised the study for important intellectual

contents. MEI and BKE confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The Research and Ethics Committee at the College of Medicine, University of Bisha (Bisha, Saudi Arabia) reviewed and approved the present study protocol.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sheng WH, Badal RE and Hsueh PR; SMART Program: Distribution of extended-spectrum β-lactamases, AmpC β-lactamases, and carbapenemases among *Enterobacteriaceae* isolates causing intra-abdominal infections in the Asia-Pacific region: Results of the study for Monitoring Antimicrobial Resistance Trends (SMART). Antimicrob Agents Chemother 57: 2981-2988, 2013.
2. Manyahi J, Moyo SJ, Tellevik MG, Ndugulile F, Urassa W, Blomberg B and Langeland N: Detection of CTX-M-15 beta-lactamases in *Enterobacteriaceae* causing hospital- and community-acquired urinary tract infections as early as 2004, in Dar es Salaam, Tanzania. BMC Infect Dis 17: 282, 2017.
3. Jiang W, Yang W, Zhao X, Wang N and Ren H: *Klebsiella pneumoniae* presents antimicrobial drug resistance for β-lactam through the ESBL/PBP signaling pathway. Exp Ther Med 19: 2449-2456, 2020.
4. Hassan MI, Alkharsah KR, Alzahrani AJ, Obeid OE, Khamis AH and Diab A: Detection of extended spectrum beta-lactamases-producing isolates and effect of AmpC overlapping. J Infect Dev Ctries 7: 618-629, 2013.
5. Marie MA, John J, Krishnappa LG and Gopalkrishnan S: Molecular characterization of the β-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* from a tertiary care hospital in Riyadh, Saudi Arabia. Microbiol Immunol 57: 805-810, 2013.
6. Yezli S, Shibl AM and Memish ZA: The molecular basis of β-lactamase production in Gram-negative bacteria from Saudi Arabia. J Med Microbiol 64: 127-136, 2015.
7. Parajuli NP, Maharjan P, Joshi G and Khanal PR: Emerging Perils of Extended Spectrum β-Lactamase Producing *Enterobacteriaceae* Clinical Isolates in a Teaching Hospital of Nepal. BioMed Res Int 2016: p1-7, 2016.
8. Tawfik AF, Alswailem AM, Shibl AM and Al-Agamy MHM: Prevalence and genetic characteristics of TEM, SHV, and CTX-M in clinical *Klebsiella pneumoniae* isolates from Saudi Arabia. Microb Drug Resist 17: 383-388, 2011.
9. Dirar MH, Bilal NE, Ibrahim ME and Hamid ME: Prevalence of extended-spectrum β-lactamase (ESBL) and molecular detection of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genotypes among *Enterobacteriaceae* isolates from patients in Khartoum, Sudan. Pan Afr Med J 37: 213, 2020.
10. Shahcheraghi F: Haleh Moezi MMF: Distribution of TEM and SHV beta-lactamase genes among *Klebsiella pneumoniae* strains isolated from patients in Tehran. Med Sci Monit 13: BR247-BR251, 2007.
11. Somily AM, Habib HA, Absar MM, Arshad MZ, Manneh K, Al Subaie SS, Al Hedaithy MA, Sayyed SB, Shakoor Z and Murray TS: ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care hospital in Saudi Arabia. J Infect Dev Ctries 8: 1129-1136, 2014.
12. Nedjai S, Barguigua A, Djahmi N, Jamali L, Zerouali K, Dekhil M and Timinouni M: Prevalence and characterization of extended spectrum beta-lactamase-producing *Enterobacter cloacae* strains in Algeria. J Infect Dev Ctries 7: 804-811, 2013.
13. Zowawi HM, Balkhy HH, Walsh TR and Paterson DL: β-lactamase production in key gram-negative pathogen isolates from the Arabian Peninsula. Clin Microbiol Rev 26: 361-380, 2013.
14. Alyamani EJ, Khiyami AM, Booq RY, Majrashi MA, Bahwerth FS and Rechkina E: The occurrence of ESBL-producing *Escherichia coli* carrying aminoglycoside resistance genes in urinary tract infections in Saudi Arabia. Ann Clin Microbiol Antimicrob 16: 1, 2017.
15. Al-Agamy MHM, Shibl AM and Tawfik AF: Prevalence and molecular characterization of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in Riyadh, Saudi Arabia. Ann Saudi Med 29: 253-257, 2009.
16. Hassan H and Abdalhamid B: Molecular characterization of extended-spectrum beta-lactamase producing *Enterobacteriaceae* in a Saudi Arabian tertiary hospital. J Infect Dev Ctries 8: 282-288, 2014.
17. Al-Qahtani AA, Al-Agamy MH, Ali MS, Al-Ahdal MN, Aljohi MA and Shibl AM: Characterization of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* from Riyadh, Saudi Arabia. J Chemother 26: 139-145, 2014.
18. Al-Agamy MH, Shibl AM, Hafez MM, Al-Ahdal MN, Memish ZA and Khubnani H: Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* in Riyadh: Emergence of CTX-M-15-producing *E. coli* ST131. Ann Clin Microbiol Antimicrob 13: 4, 2014.
19. Alqasim A, Abu Jaffal A and Alyousef AA: Prevalence of Multidrug Resistance and Extended-Spectrum β-lactamase Carriage of Clinical Uropathogenic *Escherichia coli* Isolates in Riyadh, Saudi Arabia. Int J Microbiol 2018: 3026851, 2018.
20. Ibrahim ME, Abbas M, Al-Shahrai AM and Elamin BK: Phenotypic Characterization and Antibiotic Resistance Patterns of Extended-Spectrum β-Lactamase- and AmpC β-Lactamase-Producing Gram-Negative Bacteria in a Referral Hospital, Saudi Arabia. Can J Infect Dis Med Microbiol 2019: 6054694, 2019.
21. Al-Garni SM, Ghonaim MM, Ahmed MMM, Al-Ghamdi AS and Ganai FA: Risk factors and molecular features of extended-spectrum beta-lactamase producing bacteria at southwest of Saudi Arabia. Saudi Med J 39: 1186-1194, 2018.
22. Al Bshabshe A, Joseph MRP, Al Hussein A, Haimour W and Hamid ME: Multidrug resistance Acinetobacter species at the intensive care unit, Aseer Central Hospital, Saudi Arabia: A one year analysis. Asian Pac J Trop Med 9: 903-908, 2016.
23. Elabd FM, Al-Ayed MSZ, Asaad AM, Alsareii SA, Qureshi MA and Musa HA-A: Molecular characterization of oxacillinases among carbapenem-resistant *Acinetobacter baumannii* nosocomial isolates in a Saudi hospital. J Infect Public Health 8: 242-247, 2015.
24. Almaghrabi MK, Joseph MRP, Assiry MM and Hamid ME: Multidrug-Resistant *Acinetobacter baumannii*: An Emerging Health Threat in Aseer Region, Kingdom of Saudi Arabia. Can J Infect Dis Med Microbiol 2018: 9182747, 2018.
25. Ibrahim ME: High antimicrobial resistant rates among Gram-negative pathogens in intensive care units. A retrospective study at a tertiary care hospital in Southwest Saudi Arabia. Saudi Med J 39: 1035-1043, 2018.
26. Jarlier V, Nicolas MH, Fournier G and Philippon A: Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: Hospital prevalence and susceptibility patterns. Rev Infect Dis 10: 867-878, 1988.
27. Clinical and Laboratory Standards Institute (CLSI): Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Seventh Informational Supplement. Document M100-S27. CLSI, Wayne, PA, 2017.
28. Dallenne C, Da Costa A, Decré D, Favier C and Arlet G: Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother 65: 490-495, 2010.
29. Mashwal FA, El Safi SH, George SK, Adam AA and Jebakumar AZ: Incidence and molecular characterization of the extended spectrum beta lactamase-producing *Escherichia coli* isolated from urinary tract infections in Eastern Saudi Arabia. Saudi Med J 38: 811-815, 2017.
30. Gupta V, Ye G, Olesky M, Lawrence K, Murray J and Yu K: Trends in resistant *Enterobacteriaceae* and *Acinetobacter* species in hospitalized patients in the United States: 2013-2017. BMC Infect Dis 19: 742, 2019.

31. Alyamani EJ, Khiyami MA, Booq RY, Alnafjan BM, Altammami MA and Bahwerth FS: Molecular characterization of extended-spectrum beta-lactamases (ESBLs) produced by clinical isolates of *Acinetobacter baumannii* in Saudi Arabia. Ann Clin Microbiol Antimicrob 14: 38, 2015.
32. Abujnah A.A, Zorgani A, Sabri MAM, Mohammady HE, Khalek RA and Ghenghesh KS: Multidrug resistance and extended-spectrum β-lactamases genes among *Escherichia coli* from patients with urinary tract infections in Northwestern Libya. Libyan J Med 10: 26412, 2015.
33. Zeynudin A, Pritsch M, Schubert S, Messerer M, Liegl G, Hoelscher M, Belachew T and Wieser A: Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β-lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. BMC Infect Dis 18: 524, 2018.
34. Dirar M, Bilal N, Ibrahim ME and Hamid M: Resistance Patterns and Phenotypic Detection of β -lactamase Enzymes among *Enterobacteriaceae* Isolates from Referral Hospitals in Khartoum State, Sudan Bacterial isolates and antimicrobial susceptibility testing. Cureus 12: e7260, 2020.
35. Woodford N: Rapid characterization of beta-lactamases by multiplex PCR. Methods Mol Biol 642: 181-192, 2010.
36. Bindayna K, Khanfar HS, Senok AC and Botta GA: Predominance of CTX-M genotype among extended spectrum beta lactamase isolates in a tertiary hospital in Saudi Arabia. Saudi Med J 31: 859-863, 2010.
37. Olowo-Okere A, Ibrahim YKE and Olayinka BO: Molecular characterisation of ESBL producing Gram-negative bacterial isolates from surgical wounds of patients at a hospital in north central Nigeria. J Glob Antimicrob Resist 14: 85-89, 2018.
38. Kpoda DS, Ajayi A, Somda M, Traore O, Guessennd N, Ouattara AS, Sangare L, Traore AS and Dosso M: Distribution of resistance genes encoding ESBLs in *Enterobacteriaceae* isolated from biological samples in health centers in Ouagadougou, Burkina Faso. BMC Res Notes 11: 471, 2018.
39. Yasir M, Ajlan AM, Shakil S, Jiman-Fatani AA, Almasaudi SB, Farman M, Baazeem ZM, Baabdullah R, Alawi M, Al-Abdullah N, *et al*: Molecular characterization, antimicrobial resistance and clinico-bioinformatics approaches to address the problem of extended-spectrum β-lactamase-producing *Escherichia coli* in western Saudi Arabia. Sci Rep 8: 14847, 2018.
40. Al-Agamy MH, El Mahdy TS and Shibl AM: Fecal Colonization with Extended-Spectrum Beta-Lactamase and AmpC-Producing *Escherichia coli*. BioMed Res Int 2016: 3704150, 2016.
41. Barguigua A, El Otmani F, Talmi M, Bourjilat F, Haouzane F, Zerouali K and Timinouni M: Characterization of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from the community in Morocco. J Med Microbiol 60: 1344-1352, 2011.
42. Habeeb MA, Sarwar Y, Ali A, Salman M and Haque A: Rapid emergence of ESBL producers in *E. coli* causing urinary and wound infections in Pakistan. Pak J Med Sci 29: 540-544, 2013.
43. Ning BT, Zhang CM, Liu T, Ye S, Yang ZH and Chen ZJ: Pathogenic analysis of sputum from ventilator-associated pneumonia in a pediatric intensive care unit. Exp Ther Med 5: 367-371, 2013.