

Risk factors and molecular features of extended-spectrum beta-lactamase producing bacteria at southwest of Saudi Arabia

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ABSTRACT

الأهداف: تحديد عوامل الخطورة، نسبة الانتشار وأنواع الجينات للبكتيريا المفرزة لانزيمات البيتا لاكتاميز واسعة الطيف كمسبب للعدوى بمستشفى الملك عبدالعزيز التخصصي، الطائف، المملكة العربية السعودية.

الطريقة: أجريت هذه الدراسة الاستيعابية خلال الفترة من فبراير 2017م إلى يناير 2018م. جميع العينات تم جمعها من مستشفى الملك عبدالعزيز التخصصي، الطائف، المملكة العربية السعودية ومن ثم الكشف عن بكتيريا المفرزة للبيتا لاكتاميز واسعة الطيف باستخدام جهاز MicroScan Walkaway وطرق الفحص الروتينية واختبار تآزر القرص المزدوج. تم الكشف أيضاً عن أنواع الانزيمات باستخدام تقنية PCR.

النتائج: وجدنا 351 عينة من إجمالي العينات السالبة لصبغة جرام والبالغ عددها 1151 (30.5%) عينة كانت قادرة على إفراز إنزيمات البيتا لاكتاميز واسعة الطيف. كانت *Escherichia coli* السائدة (62%) يليها *Klebsiella pneumoniae* نسبة (23.6%). شكلت عينات البول النسبة الأكبر من العينات (62.5%). الحالات الايجابية للبكتيريا المنتجة لانزيمات البيتا لاكتاميز من القسم الطبي للنساء كانت الأعلى (20.2%). عوامل الخطورة المرتبطة بانتشار عدوى هذه البكتيريا شملت أمراض الكلى، السكري، أمراض الجهاز التنفسي وأمراض القلب. مجموعة Carbapenems هي الملائمة لعلاج المرضى المصابين بهذه البكتيريا. أظهرت النتائج ارتفاع انتشار أنزيم blaCTX-M نسبة (87%) يليه، blaTEM نسبة (74.9%)، ثم blaSHV نسبة (29.4%).

الخلاصة: هذه الدراسة تضيف العديد من المعلومات عن مدى انتشار البكتيريا المفرزة لانزيمات البيتا لاكتاميز واسعة الطيف بين مرضى مستشفى الملك عبدالعزيز التخصصي بالطائف. بالإضافة إلى تحديد أنواع الانزيمات المفرزة من هذه البكتيريا.

Objectives: To explore the risk factors, the prevalence rate, and gene types of extended-spectrum beta-lactamase (ESBL)-producing bacteria as the causative agents of infection at King Abdulaziz Specialist Hospital (KAASH), Taif, Kingdom of Saudi Arabia.

Methods: This was a retrospective study conducted during the period between February 2017 and January 2018. All samples obtained from the KAASH were analyzed. The MicroScan Walkaway System, bacteriological

examination and double disk synergy tests were used to detect ESBL-producing bacteria. To identify ESBL genes, the polymerase chain reaction (PCR) technique was used.

Results: The ESBL phenotype was detected in 351 of 1151 isolates (30.5%); *Escherichia coli* (*E. coli*) (62.7%) and *Klebsiella pneumoniae* (*K. pneumoniae*) (23.6%) were the most prevalent. The highest proportion of ESBL specimens was found in urine (62.5%), and these organisms were mainly isolated from the female medical ward (20.2%). Based on the statistical analysis, lung diseases, renal diseases, diabetes and heart diseases contributed to the spread of ESBL infections. Amikacin, imipenem, meropenem and tigecycline were found to be effective in overcoming ESBL infections; however, these antibiotics may be inappropriate for new strains of *K. pneumoniae*. The distribution of the blaCTX-M gene was high (87%), compared with blaTEM (74.9%) and blaSHV (29.4%).

Conclusion: These data provide new epidemiological information about the prevalence of ESBL-producing organisms among patients in KAASH, Taif, Saudi Arabia. In addition, this study identified the clonal nature of isolated *E. coli* and *K. pneumoniae*.

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Today, one challenge faced by the World Health Organization (WHO) is extended-spectrum beta-lactamase (ESBL)-producing bacteria. Rates of ESBL production among *Enterobacteriaceae* range between 6-38% in Kingdom of Saudi Arabia, with the highest frequency recorded in the central regions.¹ Excessive use of third-generation cephalosporins, which are commonly used as an empiric and definitive therapy in hospitals, are expected to trigger the emergence and spread of ESBL producers.²

There are several risk factors that may contribute to the prevalence of ESBL infection among patients and healthcare workers. Studies aimed at identifying these factors, and the extent of their association with ESBL-producing bacteria, have largely agreed on the contributions of certain factors, such as hospital admission and the previous use of antibiotics, but have conflicting results with respect to the impact of other factors such as gender, age, and chronic disease (namely, diabetes and renal disease). Therefore, factors linked with the spread of ESBL infection remain controversial issues and require further research to determine the relationship between these factors and the acquisition of ESBL infection.

Extended-spectrum beta-lactamases-producing bacteria have the ability to secrete common enzymes, such as blaCTX-M, blaTEM and blaSHV, and almost 450 variants of these enzymes have been detected.³ This is undoubtedly due to significant advancements in gene detection methods, such as polymerase chain reaction (PCR), that provides very accurate results. Extended-spectrum beta-lactamases producing organisms break the beta-lactam ring, which leads to resistance to antimicrobials, such as cephalosporins and monobactams.⁴ In addition, extensive use of these drugs leads to mutations of ESBL genes and the emergence of ESBL infection. The remarkable proliferation of such bacteria globally is causing an increase in the proportion of infected patients in the community and hospitals, as well as higher mortality rates. Various studies have indicated that the spread of such bacteria, which are capable of secreting these enzymes, might be related to the geographic region. For instance, in Saudi Arabia, the prevalence of blaSHV in *Klebsiella pneumoniae*

(*K. pneumoniae*) isolates was approximately 78% in Al-Ahasa and less than 14% in Mecca hospitals.^{5,6}

According to several researches, blaCTX-M-15 is predominant, compared with other genes.⁷ Clonal proliferation and genetic elements have been considered causes of this spread.⁸ This retrospective study aims to identify the risk factors of infection caused by ESBL-producing bacteria at the KAASH, Taif, Kingdom of Saudi Arabia and to identify the different types of ESBL enzymes that are found using PCR methods.

Methods. This was a retrospective study and the isolates analyzed were from the KAASH in Taif, Kingdom of Saudi Arabia which has a 500-bed capacity. It is a referral hospital and serves as a teaching hospital for Taif University Medicine College, Taif, Saudi Arabia. This study was conducted between February, 2017 and January, 2018. Urine, blood, sputum, swabs and other clinical specimens were cultured on blood agar and MacConkey agar (Saudi prepared media laboratory [SPML]). Patients with gram-negative bacteria (GNB) infections were recorded. Only one sample from each patient was considered. Identification of the organisms was performed using the Microscan Walkaway system (Beckman Coulter, USA), according to the manufacturer's instructions. Other tests such as analytic profile index (20EAPI-20E) and E-test (bioMérieux, France), and double disc synergy test (DDST) were also performed if needed.

The ESBL production was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines of 2012.⁹ Mueller-Hinton agar (SPML) plates were inoculated with the test organisms. Ceftazidime and co-amoxiclav discs were placed 30 mm apart (centre to centre) and incubated at 37°C for 24 hours. Enhancement of the inhibition zone in the area between the discs indicated a positive result. The E-test (bioMérieux, France) was used to confirm the ESBL result. The specimen was spread onto a Mueller-Hinton agar plate. If the ratio of the minimum inhibitory concentration of co-amoxiclav to that of ceftazidime was more than 8, the result was considered positive. Routine susceptibility was determined using the disc-diffusion method on Mueller-Hinton agar, as recommended by the CLSI. To ensure quality control, *Escherichia coli* (*E. coli*) (ATCC 25922) and *K. pneumoniae* (ATCC 700603) ESBL-producing strains were used as controls for the antimicrobial susceptibility tests.

A genomic DNA purification kit (Jena Bioscience GmbH, Germany) was used to purify ESBL-producing *E. coli* (n=209, 59.5%) and *K. pneumoniae* (n=90, 25.6%) isolates. The PCR amplification of

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blaCTX-MF was performed using the following primers: (5'-TTAGGAARTGTGCCGCTGYA-3') and blaCTX-MR was: 5'-CGATATCGTTGGTGGTRCCAT-3' with an amplicon size of 688bp.¹⁰ Primers used for blaTEM-C was: 5'-ATCAGCAATAAACCAGC-3' and blaTEM-H was: 5'-CCCCGAAGAACGTTTTTC-3' with an amplicon size of 516bp.¹¹ Primers used for blaSHV-F was: 5'-AGGATTGACTGCCTTTTTG-3' and blaSHV-R was 5'-ATTTGCTGATTTTCGCTCG-3' with an amplicon size of 392pb.¹¹ For amplification, 2 µl template DNA (around 50 ng) was added to 23 µl reaction mixture, which contained 12.5 µl GoTaq green master mix (Promega, USA), 0.5 µl of each primer and 10 µl nuclease-free water. A C1000 Touch thermal cycler (Bio-Rad, Germany) was used to complete the PCR reaction as follows: denaturation at 94°C for 5 minutes was followed by 35 cycles of denaturation at 94°C for 45 seconds, then annealing for 45 seconds (at 50°C for blaTEM and blaSHV and at 60°C for blaCTX-M), extension at 72°C for 60 seconds and a final extension at 72°C for 7 minutes. The polymerase chain reaction amplicons were separated by electrophoresis on a 1.5% agarose gel in 1X TAE (Tris/Acetate/EDTA) buffer containing 0.5ug/ml ethidium bromide. The last step was photographed under an ultraviolet lamp (Bio-Rad Gel Doc 2000 device).

Statistical analysis. Microsoft Software 2010 was used in the analysis of the results, which are presented as frequencies or percentages. This software was also used to determine the *p*-values of different characteristics of patients who were infected with ESBL.

Results. A total of 351 ESBL-producing bacteria were isolated from 1151 GNB (inpatients 87.7% and outpatients 12.3%). The majority of the ESBL-producing isolates were *E. coli* (220/351, 62.7%), followed by *K. pneumoniae* (83/351, 23.6%) and *Proteus mirabilis* (38/351, 10.8%). Other microbes that can secrete these enzymes such as *Enterobacter cloacae*, *Morganella morganii*, *Serratia marcescens*, *Klebsiella oxytoca*, *Providencia stuartii* and *Citrobacter amalonaticus* were less widespread in the hospital environment (10/351, 2.8%) (Figure 1).

Extended-spectrum beta-lactamase isolates were mainly recovered from urine in high percentages of 62.1% (218/351). Other ESBL isolates were recovered from swabs (49, 14%), followed in decreasing order by blood (36, 10.2%), sputum (32, 9.1%) and other (16, 4.6%).

Table 1 compares the percentages of ESBL among males, females, and different age groups. Remarkable differences between males and females were found, with ESBL infection presenting in more females than males (59.8%, 210/351). Furthermore, females presented almost the same percentage of non-ESBL producers as ESBL producers at 54.8%. The proportion of females aged ≥80 years among the ESBL and non-ESBL producers was lower than that of males in the same age bracket and was 6% and 8%. For males, the number of non-ESBL producer bacteria outnumbered females by roughly 2-one in the age groups of 0-9 and 10-19 years old (13%, 47/362 and 6.6%, 29/438). In addition, the spread of ESBL infection among males aged 10-19 showed the lowest level among either gender with nearly 2% (6/351).

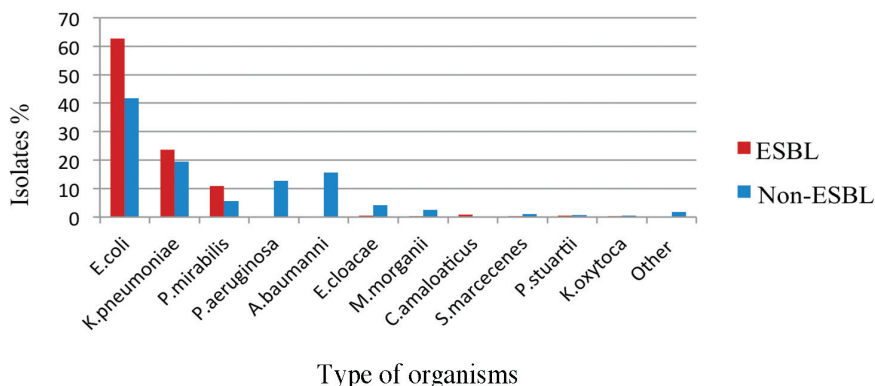


Figure 1 - Distribution of extended-spectrum beta-lactamase (ESBL) and non-ESBL isolates according to species. *E. coli* - *Escherichia coli*, *K. pneumoniae* - *Klebsiella pneumoniae*, *P. mirabilis* - *Proteus mirabilis*, *P. aeruginosa* - *Pseudomonas aeruginosa*, *A. baumannii* - *Acinetobacter baumannii*, *E. cloacae* - *Enterobacter cloacae*, *M. morganii* - *Morganella morganii*, *C. amalonaticus* - *Citrobacter amalonaticus*, *S. marcescens* - *Serratia marcescens*, *P. stuartii* - *Providencia stuartii*, *K. oxytoca* - *Klebsiella oxytoca*

Table 1 - Age distribution of patients with ESBL producers and non-ESBL.

Age group (years)	ESBL producers (n=351)		Non-ESBL producers (n=800)	
	Male	Female	Male	Female
0-9	8 (5.7)	10 (4.8)	26 (7.3)	12 (2.7)
10-19	6 (4.3)	9 (4.3)	21 (5.8)	17 (3.9)
20-29	15 (10.6)	30 (14.3)	44 (12.2)	82 (18.8)
30-39	17 (12.1)	38 (18.1)	39 (10.8)	65 (14.9)
40-49	15 (10.6)	17 (8.1)	36 (9.9)	52 (11.9)
50-59	14 (9.9)	22 (10.5)	38 (10.5)	43 (9.8)
60-69	20 (14.2)	26 (12.4)	37 (10.2)	59 (13.5)
70-79	15 (10.6)	37 (17.6)	50 (13.8)	53 (12.1)
80-89	22 (15.6)	14 (6.7)	48 (13.3)	39 (8.9)
90-99	9 (6.4)	7 (3.3)	23 (6.4)	16 (3.7)
Total	141	210	362	438

Values are presented as number and percentage (%), ESBL - extended-spectrum beta-lactamase

Extended-spectrum beta-lactamase infection was the most common in the female medical ward, with approximately 21% of the total isolates. The spread of ESBL-producing bacteria among patients in the male surgical ward was also high (17.7%), compared with other wards. In contrast, ESBL infection was less spread among patients in the male medical ward-high dependency unit and occupational therapy and had, the same rate of 0.6%. The male medical ward, female surgical ward and intensive care unit (ICU) were other sections in which ESBL infection spread among patients, with about 13% (Table 2).

The total number of non-Saudi patients was 24, and all of these patients were from Asia or Africa (13.5%, 155/1151). The ESBL infection accounted for 9.7% of the total isolates (34/351) and most of these patients were Yemeni (29.4%) or Pakistani (14.7%).

To determine the risk factors that might lead to ESBL infection among patients, the most important characteristics of patients were divided into several variables (Table 3). These variables included age, gender, underlying diseases and the use of certain antibiotics (such as gentamicin, nitrofurantoin and norfloxacin). In addition, the number of patients with ESBL infection was associated with the presence or absence of these characteristics. For example, kidney diseases accounted for roughly 28% (98/351) of the total number of patients infected with ESBL, whereas patients with non-ESBL were 16.9% (135/800).

Table 2 - Distribution of ESBL-producing and non-ESBL bacteria according to hospital wards.

Hospital ward	ESBL producers	Non-ESBL producers
Female medical ward	71 (20.2)	97 (12.1)
Male medical ward	47 (13.4)	78 (9.8)
Intensive care unit	43 (12.3)	94 (11.8)
Male surgical ward	62 (17.7)	147 (18.4)
Female surgical ward	47 (13.4)	75 (9.4)
Antenatal care	13 (3.7)	6 (0.8)
Gynecology	13 (3.7)	45 (5.6)
Outpatient ward	18 (5.1)	124 (15.5)
Coronary care unit	3 (0.9)	7 (0.9)
Male surgical-high dependency unit	7 (1.9)	10 (1.3)
Female medical-high dependency unit	6 (1.7)	18 (2.3)
Post-operative observation ward	4 (1.1)	7 (0.9)
Female surgical-high dependency unit	5 (1.4)	14 (1.8)
Emergency	4 (1.1)	0
Occupational therapy	2 (0.6)	0
Anteroom	4 (1.1)	44 (5.5)
Male medical ward-high dependency unit	2 (0.6)	26 (3.2)
Other	0	8 (1.0)
Total	351	800

Values are presented as number and percentage (%), ESBL - extended-spectrum beta-lactamase.

Table 3 - Potential risk factors and clinical traits for ESBL-producing bacterial infections.

Parameter	ESBL (n=351)	Non-ESBL (n=800)	P-value
<i>Gender</i>			
Female	210 (59.8)	438 (54.8)	0.8
Male	141 (40.2)	362 (45.3)	0.2
<i>Age ≥60 years</i>			
Female	84 (23.9)	167 (20.9)	0.1
Male	66 (18.8)	158 (19.8)	0.4
Diabetes mellitus/diabetes foot	22 (6.3)	32 (2.9)	0.02
Kidney disease	98 (27.9)	135 (16.9)	0.0001
Respiratory diseases	19 (5.4)	23 (2.9)	0.04
Heart diseases	45 (12.8)	90 (11.3)	0.003
Central nervous system	3 (0.9)	4 (0.5)	1
Cancer	5 (1.4)	11 (1.4)	0.8
Gynaecology and obstetrics	13 (3.7)	7 (0.9)	0.2
Gentamicin	94 (26.8)	69 (8.6)	0.7
Nitrofurantoin	38 (10.8)	26 (3.3)	0.5
Norfloxacin	165 (47.0)	90 (11.3)	1.0

Values are presented as number and percentage (%), ESBL - extended-spectrum beta-lactamase.

Several clinical features are associated with the respiratory system, such as community-acquired pneumonia and chronic obstructive pulmonary disease (COPD). These diseases accounted for around 5% of patients with an ESBL infection, and were reported among approximately 3% of the patients with non-ESBL infections. This finding indicates that there is an important relationship between some respiratory diseases and ESBL infection ($p=0.04$). Furthermore, heart diseases included 6 variables in this study. Complete heart disease, post-cardiac arrest, stroke, hypertension, ischemic heart disease and atrial fibrillation indicated an important affiliation with ESBL infection ($p=0.003$).

For gender, the proportion of women and men with ESBL infections indicate that gender is a non-significant factor for acquiring this infection ($p=0.7$ and $p=0.2$). In addition, age did not have an effect on ESBL infection among different age groups ($p>0.05$). It was clear from the results that patients with diabetes were most likely to acquire ESBL than were other patients ($p=0.02$). However, there was no significant link between ESBL infection and other comorbid conditions, such as central nervous system and cancer ($p=1$ and $p=0.8$). The antibiotics, gentamicin, nitrofurantoin and norfloxacin did not have a significant connection with ESBL infection ($p>0.05$). In this study, some antibiotics were used to test the sensitivity of ESBL-producing *E.coli*

and *K.pneumoniae*, which accounted for around 86% of the total ESBL-producing bacteria. The penicillin and cephalosporin groups failed to treat such organisms, and had a resistance percentage of 100% (Table 4). However, several antimicrobials had a large effect in overcoming ESBL infections with rates ranging from 80-100%, such as amikacin, imipenem, meropenem and tigecycline. In addition, some antimicrobials had the ability to treat ESBL-producing *E.coli* at a greater percentage than ESBL-producing *K.pneumoniae*. For instance, nitrofurantoin was a significant drug for curing *E.coli* (susceptibility [S]=95%); however, it was not an appropriate choice for patients infected with *K.pneumoniae* (S=40%).

Both organisms were able to resist norfloxacin and ciprofloxacin with a high rate for *E.coli* (77% and 71%) and a low percentage for *K.pneumoniae* (32% and 48%). Furthermore, a combination of medication, such as piperacillin/tazobactam, was effective for treating ESBL-producing *E.coli* at 87%, yet was less effective for treating ESBL infection caused by *K. pneumoniae* at 75%.

Amoxicillin/clavulanate is another example drug combination that was used to treat ESBL infection; however, the results of susceptibility of ESBL-producing *E.coli* and *K. pneumoniae* indicated that this antimicrobial was not suitable for such infection.

The results indicated that the level of blaCTX-M secretion was high compared with blaTEM and blaSHV (87%, 75%, and 29%). *Klebsiella pneumoniae* could produce 2 types of enzymes (blaCTX-M and blaTEM) at a higher rate than *E.coli* (Figure 2). The blaSHV gene production by *K.pneumoniae* (57%) was also about 3 times greater than the number of *E.coli*. Also, blaCTX-M, blaTEM, and blaSHV were secreted together, equally by both organisms at nearly 17%.

Table 4 - Antimicrobial susceptibility patterns of ESBL-producing *E.coli* and *K.pneumoniae* isolates.

Antimicrobial agent	<i>E. coli</i> (n=220)			<i>K. pneumoniae</i> (n=83)		
	S	I	R	S	I	R
Amikacin	96%	3%	1%	83%	7%	10%
Amoxicillin/clavulanate	58%	33%	9%	43%	33%	24%
Ampicillin	0	0	100%	0	0	100%
Cefazolin	0	0	100%	0	0	100%
Cefepime	0	0	100%	0	0	100%
Cefotaxime	0	0	100%	0	0	100%
Aztreonam	0	0	100%	0	0	100%
Ceftazidime	0	0	100%	0	0	100%
Cefuroxime	0	0	100%	2%	0	98%
Ciprofloxacin	29%	0	71%	33%	19%	48%
Gentamicin	68%	0	32%	52%	4%	44%
Imipenem	100%	0	0	98%	2%	0
Meropenem	100%	0	0	99%	0	1%
Nitrofurantoin	95%	4%	1%	40%	21%	39%
Norfloxacin	21%	2%	77%	55%	13%	32%
Piperacillin/tazobactam	87%	10%	3%	75%	10%	15%
Tigecycline	100%	0	0	81%	11%	8%

ESBL - extended-spectrum beta-lactamase, *E. coli* - *Escherichia coli*,
K. pneumoniae - *Klebsiella pneumoniae*

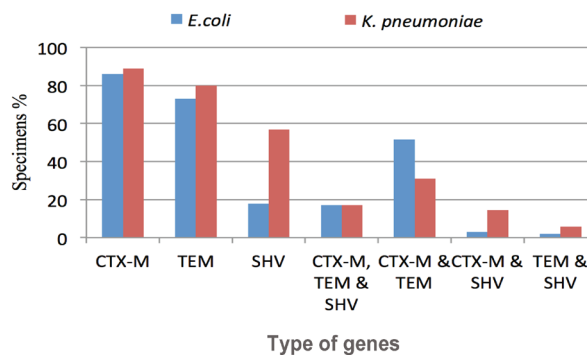


Figure 2 - The extended-spectrum beta-lactamase (ESBL) genes distribution among *Escherichia coli*, and *Klebsiella pneumoniae*.

Escherichia coli released both blaCTX-M and blaTEM more so than *K.pneumoniae*, at 51%.

For secreting blaCTX-M and blaSHV together, *K.pneumoniae* was able to secrete these enzymes with a higher percentage than *E.coli* at 13% and 3%. The blaTEM and blaSHV genes were the lowest of that ESBL enzymes that were produced by *K.pneumoniae* and *E.coli* at the same time (<6%). In general, *K.pneumoniae* had the ability to secrete the 3 enzymes in higher amounts than *E.coli*, whether they were together or alone, except for the production blaCTX-M and blaTEM simultaneously.

Discussion. Over the past 10 years, ESBL infections have spread throughout the different regions of Kingdom of Saudi Arabia with a high rate of 36%.¹² Within the Western Kingdom of Saudi Arabia region, high ESBL prevalence has been reported among patients in Mecca (30%) and in Jeddah (20%).^{6,13} In the present study, the prevalence of ESBL producers among gram-negative bacteria was 351 of 1151 (30.4%), which corresponds to the findings of Al Mously et al,¹⁴ in 2016 (27.4%). However, different results were reported for ESBL infection in other areas of Kingdom of Saudi Arabia, such as in the Central and the Eastern regions, with rates of 6.3% and 23%.^{15,16}

In this study, infection with ESBL-producing *E.coli* (62.7%) was the most common, followed by *K.pneumoniae* (23.6%), *P.mirabilis* (10.8%) and others with lower rates (2.8%). Similar findings were reported by Elhassan et al,¹¹ which revealed that *E.coli* and *K.pneumoniae* were detected at high rates of 77.6% and 22.4%. Although *Paeruginosa* was recorded as an ESBL-producing bacterial species with a prevalence of 25.9% in the Western region (Mecca) by Ahmed et al,¹⁷ this might have been related to Umrah or Hajj; all *Paeruginosa* specimens in our study were non-ESBL producers.

In this study, 59.8% of positive ESBL samples were from females, compared with 40.2% from males, which is comparable to the results published by Tham et al,¹⁸ 2013 (women, 77%), but differs from other studies that found that the majority of ESBL patients were male.¹⁹ The proportion of patients aged <60 years was greater than that of patients aged ≥60 years at 57.3% and 42.7%. However, there was no significant association between age and ESBL infection ($p>0.05$). The same finding was reported by Saudi researchers and others.^{12,20} In addition, non-Saudis who were infected with ESBL producers accounted for 9.7% of positive samples, and most of these patients were Yemeni (29.4%), followed by Pakistani (14.7%). These results might be due to the large proportion of these nationalities in Taif, Kingdom

of Saudi Arabia. In contrast, the study by Zaman et al,¹³ 2015 (in Mecca) indicates that the distribution of Saudi patients are less than that of non-Saudi patients: 32.3% and 67.7%. These results might have been affected by the population diversity that characterizes this region.

Regarding specimen type, urine accounted for the largest proportion of specimens positive for ESBL producers (218/351, 62.1%). Furthermore, ESBL producers were isolated from swabs (14%), blood (10.3%) and sputum (9.1%). Other researchers showed similar findings, with urine accounting for approximately 60% of the samples.^{11,12} Extended-spectrum beta-lactamase producers were primarily isolated from the female medical ward (20.2%), male surgical ward (17.7%), and male medical ward and female surgical ward (13.4%) and ICU (12.3%). These findings differ from local and global studies in which the highest percentages of ESBL producers were isolated from the ICU.^{7,21}

In the present study, the lowest rates of ESBL infections were in female patients aged 90-99 years (3.3%), while the highest percentages were from female patients aged 30-39 years (18%). A study in 2014 reported that patients aged ≤18 years were less likely to be infected with ESBL bacteria.¹⁵ During the period 2006-2011, Kassakian and Mermel studied 321 ESBL specimens and found that the age of patients was >65 years and that approximately 74% were female.²² However, older age and gender were not significantly correlated with ESBL infection in the present study ($p>0.05$), and these results correspond with data reported by other investigations ($p=0.3$ and $p=0.4$).^{12,23}

Kidney dysfunction is a serious disease that, is associated with ESBL-producing Enterobacteriaceae. Several studies indicate that the prevalence of ESBL-producing *E.coli* and *K.pneumoniae* among patients with renal disease was noteworthy.²⁴ In this research, the spread of ESBL-producing strains in patients caused significant renal dysfunction during various stages of the disease and, resulted in kidney failure ($p=0.0001$). These findings were consistent with those previously reported for the King Khalid University Hospital in Riyadh, Kingdom of Saudi Arabia.²⁵

Several studies have reported diabetes as a risk factor for ESBL infection. In 2016, for example, Al Yousef et al,²⁶ isolated 296 ESBL samples and found that diabetes was a risk factor associated with ESBL infection. In addition, Rahim et al,²⁷ found that diabetes was significantly linked to ESBL infection. The current study reveals that diabetes is significantly associated with ESBL infection ($p=0.02$). However, a retrospective study performed in Turkey in 2013, showed that there was no link between diabetes and ESBL infection

($p=0.3$).²⁸ Based on multivariate analysis, there were several characteristics related to lung diseases, such as COPD and respiratory disease syndrome (RDS). The percentage of lung disease cases with ESBL-producing bacteria was greater than non-ESBL at 5.4% and 2.8%. This finding reveals the important relationship between such diseases and ESBL infection ($p=0.04$). Other studies by Blom et al,²⁹ and Vijayakanthi et al,³⁰ confirmed that COPD and RDS were underlying illnesses associated with ESBL infection.

Heart failure/heart disease causes and ESBL infection have been reported in abundant scientific research. Some of these studies did not find an important connection between such diseases and ESBL organisms.¹⁸ However, the study by Quan et al,³¹ concluded that heart failure may be associated with the prevalence of ESBL *K.pneumoniae* in hospitals ($p=0.001$). In this investigation, several comorbidities related to heart diseases showed a significant link to ESBL-positive Enterobacteria ($p=0.003$). In general, the current study indicates that piperacillin/tazobactam, amikacin and nitrofurantion, have the ability to treat both ESBL and non-ESBL infections. These results correspond to those published by Khalid et al,³² who recommended the use of nitrofurantion as an oral drug against ESBL producers and other urinary pathogens. In addition, several drugs retain the ability to overcome ESBL-producing bacteria. For instance, in this data, imipenem, meropenem and tigecycline showed the capacity to cure a large range of ESBL bacteria.

Extended-spectrum beta-lactamase-producing bacteria carry highly diverse enzymes that include blaCTX-M, blaTEM, blaSHV, blaOXA, blaPER, blaVEB and blaBES.³³ The first and the second genes were more abundant than the other genes. Certain factors contribute to the prevalence of ESBL infection, such as society, health organizations and the economics of different countries.³⁴ Different studies have recorded high rates of ESBL infection in India (80%), with a large population and inadequate quality of life, and Egypt (70%), compared with 2.5% in Sweden and 5% in America. In the Middle East, this percentage varies from 16% in Lebanon to 26% in Kuwait and 38% in Kingdom of Saudi Arabia.³⁴

Furthermore, the spread of a specific type of bacteria plays an important role in increasing resistance to specific types of antibiotics. For example, blaCTX-M is associated with *E.coli* in many cases, while blaTEM and blaSHV are linked to *K.pneumoniae*, and both display resistance to the third-generation cephalosporins.³⁵ The blaOXA gene appears in *P.aeruginosa*, which has the ability to resist cloxacillin and oxacillin, but is more sensitive to the cephalosporins group.³⁶ In this study,

blaCTX-M was predominant with a high rate of 87%, followed by blaTEM (74.9%) and blaSHV (29.4%). Sharma et al,³⁷ shared this finding with, 83% for blaCTX-M and 67% for blaTEM.

Some studies have shown that certain bacteria are able to secrete 2 or 3 enzymes. For example, a study consisting of 81 ESBL-producing *E.coli* and 21 *K.pneumoniae* found that certain strains of *E.coli* have the ability to produce blaCTX-M, blaTEM and blaSHV, accounting for 13% of total secreted enzymes, while other strains of the same organism released blaTEM and blaSHV at 2.5%. *Klebsiella pneumoniae* had the same ability to produce 2 enzymes (blaTEM and blaSHV) and 3 enzymes (blaCTX-M, blaTEM and blaSHV), at 19% of total isolates.⁶ Other local investigation revealed that both blaSHV and blaTEM were released at the low level of 3.5%.¹¹ In the present study, both blaTEM and blaSHV were detected in 3% of isolates, while 17% of the isolates carried all 3 genes together, blaCTX-M, blaTEM and blaSHV. The distribution of blaSHV depends on the rate of ESBL infection caused by *K.pneumoniae*. A study by Dehshiri et al,³⁸ on the occurrence of ESBL-producing *K.pneumoniae* in patients with urinary tract infection concluded that the prevalence of blaSHV was very high (85%), compared with that of blaCTX-M and blaTEM at 30% and 16%. In the present investigation, the prevalence of blaSHV was greater in *K.pneumoniae* (56.7%) than *E.coli* (17.7%). The high prevalence of these enzymes may contribute increasing antimicrobial resistance among Enterobacteriaceae. These resistant strains are selected by overuse and misuse of antibiotics in our locality because of the high prevalence of antibiotic misuse which has been reported in Taif, Kingdom of Saudi Arabia as well as other regions in Kingdom of Saudi Arabia.³⁹

Study limitation. The PCR technique was performed to detect genotypes of 2 organisms, *E.coli* and *K.pneumoniae*. In addition, the detected blaTEM and blaHSV genes were not sequences.

In conclusion, this study highlights an important link between ESBL prevalence and certain factors such as renal disease, respiratory diseases, heart diseases and diabetes. In addition, the uncontrolled use of antibiotics has increased the resistance of ESBL-producing bacteria. Fortunately, various drugs, that include amikacin, imipenem and meropenem, retain the ability to overcome ESBL infection. However, the presence of *K.pneumoniae* in hospitals or in the community contributes to increase the distribution of the blaSHV gene, which will create more suffering for patients. Additional studies are needed to determine other

factors that may be associated with ESBL infection and to determine the appropriate solutions to reduce the effects of these factors on the spread of this infection.

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References

- Zowawi HM, Balkhy HH, Walsh TR, Paterson DL. β -Lactamase production in key gram-negative pathogen isolates from the Arabian Peninsula. *Clin Microbiol Rev* 2013; 26: 361-380.
- Hariri G, Tankovic J, Boëlle PY, Dubée V, Leblanc G, Pichereau C, et al. Are third-generation cephalosporins unavoidable for empirical therapy of community-acquired pneumonia in adult patients who require ICU admission? A retrospective study. *Ann Intensive Care* 2017; 7: 35.
- Ur Rahman S, Ali T, Ali I, Khan NA, Han B, Gao J. The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases. *Biomed Res Int* 2018; 2018: 9519718.
- Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J Biol Sci* 2015; 22: 90-101.
- Alsultan AA, Aboulmagd E, Amin TT. ESBL-producing *E. coli* and *K. pneumoniae* in Al-Ahsa, Saudi Arabia: antibiotic susceptibility and prevalence of blaSHV and blaTEM. *J Infect Dev Ctries* 2013; 7: 1016-1019.
- Asghar AH. Prevalence and potential types of extended spectrum beta-lactamase among *Escherichia coli* and *Klebsiella* species in Makkah hospitals. *Oxf Res For J* 2007; 2: 47-55.
- Fischer J, Rodríguez I, Baumann B, Guiral E, Beutin L, Schroeter A, et al. blaCTX-M-15-carrying *Escherichia coli* and *Salmonella* isolates from livestock and food in Germany. *J Antimicrob Chemother* 2014; 69: 2951-2958.
- Al-Agamy MH, Shibl AM, Hafez MM, Al-Ahdal MN, Memish ZA, 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* 2014; 13: 4.
- Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing. Wayne (PA): Clinical and Laboratory Standards Institute; 2012. p. M100-S22.
- Alzahrani AK, Faraga MM, Abbadia SH, Hassanc MM, Gaberc A, Abdel-Moneima AS. Antibiotic resistance profile and random amplification typing of β -lactamase-producing Enterobacteriaceae from the local area of Al-Taif and nearby cities in Saudi Arabia. *Asian Biomed* 2016; 10: 219-228.
- Elhassan MM, Ozbazk HA, Hemeg HA, Ahmed AA. Dissemination of CTX-M extended-spectrum β -lactamases (ESBLs) among *Escherichia coli* and *Klebsiella pneumoniae* in Al-Madenah Al-Monawwarah region, Saudi Arabia. *Int J Clin Exp Med* 2016; 9: 11051-11057.
- Kandeel A. Prevalence and risk factors of extended-spectrum β -lactamases producing Enterobacteriaceae in a general hospital in Saudi Arabia. *JMID* 2014; 4: 50-55.
- Zaman RM, Aly MM, Helmi, NR. Antimicrobial susceptibility pattern of Gram-negative bacilli isolated from a Teaching Hospital in Jeddah, Saudi Arabia. *Afr J Microbiol Res* 2015; 9: 2145-2158.
- Al Mously N, Al Arfaj O, Al Fadhil L, Mukaddam S. Antimicrobial susceptibility patterns of ESBL *Escherichia coli* isolated from community and hospital acquired urinary tract infections. *JHS* 2016; 4: 133-139.
- Somily AM, Habib HA, Absar MM, Arshad MZ, Manneh K, Al Subaie SS, et al. ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care hospital in Saudi Arabia. *J Infect Dev Ctries* 2014; 8: 1129-1136.
- Mashwal FA, El Safi SH, George SK, Adam AA, 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* 2017; 38: 811-815.
- Ahmed OB, Asghar AH, Bahwerth FS. Prevalence of ESBL gene of *Pseudomonas aeruginosa* strains isolated from Makkah hospitals, Saudi Arabia. *European Journal of Biology and Medical Science Research* 2015; 3: 12-18.
- Tham J, Odenholt I, Walder M, Andersson L, Melander E. Risk factors for infections with extended-spectrum beta-lactamase-producing *Escherichia coli* in a county of Southern Sweden. *Infect Drug Resist* 2013; 6: 93-97.
- Peerayeh SN, Rostami E, Eslami M, Rezaee MA. High Frequency of Extended-Spectrum β -Lactamase-Producing *Klebsiella pneumoniae* and *Escherichia coli* Isolates From Male Patients' Urine. *Arch Clin Infect Dis* 2016; 11: e32696.
- Denis B, Lafaurie M, Donay JL, Fontaine JP, Oksenhendler E, Raffoux E, et al. Prevalence, risk factors, and impact on clinical outcome of extended-spectrum beta-lactamase-producing *Escherichia coli* bacteraemia: a five-year study. *Int J Infect Dis* 2015; 39: 1-6.
- Singh N, Pattnaik D, Neogi DK, Jena J, Mallick B. Prevalence of ESBL in *Escherichia coli* Isolates Among ICU Patients in a Tertiary Care Hospital. *J Clin Diagn Res* 2016; 10: DC19-DC22.
- Kassakian S, Mermel LA. Changing epidemiology of infections due to extended spectrum beta-lactamase producing bacteria. *Antimicrob Resist Infect Control* 2014; 3: 9.
- Leistner R, Meyer E, Gastmeier P, Pfeifer Y, Eller C, Dem P, et al. Risk factors associated with the community-acquired colonization of extended-spectrum beta-lactamase (ESBL) positive *Escherichia coli*. an exploratory case-control study. *PLoS One* 2013; 8: e74323.
- El-Kersh TA, Marie TA, Al-Sheikh YA, Al-Kahtani YA. Prevalence and risk factors of community-acquired urinary tract infections due to ESBL-producing Gram negative bacteria in an Armed Forces Hospital in Southern Saudi Arabia. *Global Advanced Research Journal of Medicine and Medical Sciences* 2015; 4: 321-330.
- Al-Otaibi FE, Bukhari EE. Clinical and laboratory profiles of urinary tract infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in a tertiary care center in central Saudi Arabia. *Saudi Med J* 2013; 34: 171-176.
- Al Yousef SA, Younis S, Farrag E, Moussa HSH, Bayoumi FS, Ali AM. Clinical and Laboratory Profile of Urinary Tract Infections Associated with Extended Spectrum β -Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae*. *Ann Clin Lab Sci* 2016; 46: 393-400.

27. Rahim MA, Mitram P, Haque A, Zaman S, Samad T, Wasim MD, et al. Urinary Tract Infection due to Extended-Spectrum Beta-Lactamase Producing Organisms is a Risk Factor for Acute Kidney Injury among Patients with Type 2 Diabetes Mellitus. *J Medicine* 2018; 19: 40-43.
28. Kaya O, Akcam FZ, Gonen I, Unal O, Ceylan T. Risk factors for bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli* in a Turkish hospital. *J Infect Dev Ctries* 2013; 7: 507-512.
29. Blom A, Ahl J, Månsson F, Resman F, Tham J. The prevalence of ESBL-producing Enterobacteriaceae in a nursing home setting compared with elderly living at home: a cross-sectional comparison. *BMC Infect Dis* 2016; 16: 111.
30. Vijayakanthi N, Bahl D, Kaur N, Maria A, Dubey NK. Frequency and characteristics of infections caused by extended-spectrum beta-lactamase-producing organisms in neonates: a prospective cohort study. *Biomed Res Int* 2013; 2013: 756209.
31. Quan J, Zhao D, Liu L, Chen Y, Zhou J, Jiang Y, et al. High prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in community-onset bloodstream infections in China. *J Antimicrob Chemother* 2017; 72: 273-280.
32. Khalid A, Javaid U, Fatima F, Afreenish H, Maria O, Rabia A. The frequency and antimicrobial sensitivity pattern of extended spectrum -lactamase (ESBLs) producing gram negative bacilli isolated from urine in a tertiary care hospital of Pakistan. *Afr J Microbiol Res* 2013; 7: 2040-2043.
33. Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL Production Among *E. coli* and *Klebsiella* spp. Causing Urinary Tract Infection: A Hospital Based Study. *Open Microbiol J* 2017; 11: 23-30.
34. Tham J. Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae: Epidemiology, Risk Factors, and Duration of Carriage. Sweden: Department of Clinical Sciences, Lund University; 2012.
35. Sid Ahmed MA, Bansal D, Acharya A, Elmi AA, Hamid JM, Sid Ahmed AM, et al. Antimicrobial susceptibility and molecular epidemiology of extended-spectrum betalactamase-producing Enterobacteriaceae from intensive care units at Hamad Medical Corporation, Qatar. *Antimicrob Resist Infect Control* 2016; 5: 4.
36. Evans BA, Amyesb SG. OXA-Lactamases. *Clin Microbiol Rev* 2014; 27: 241-263.
37. Sharma M, Pathak S, Srivastava P. Prevalence and antibiogram of Extended Spectrum β -Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella* spp. *J Clin Diagn Res* 2013; 7: 2173-2177.
38. Dehshiri M, Khoramrooz SS, Zoladl M, Khosravani SA, Parhizgari N, Motazedian MH, et al. The frequency of *Klebsiella pneumoniae* encoding genes for CTX-M, TEM-1 and SHV-1 extended-spectrum beta lactamases enzymes isolated from urinary tract infection. *Ann Clin Microbiol Antimicrob* 2018; 17: 4.
39. Abdallah QM. Medicines use among general public in Taif, KSA. *Arch Pharma Pract* 2014; 5: 125-131.