Original Article

ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care hospital in Saudi Arabia

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Abstract

Introduction: The increasing frequency and antibiotic resistance among extended-spectrum β-lactamases (ESBLs)-producing bacteria are posing a serious threat. This study sought to investigate the frequency and antibiotic susceptibility of ESBL-producing *E. coli* and *K. pneumoniae* at a tertiary care hospital.

Methodology: Data were collected from samples sent to the microbiology laboratory between 2006 and 2010 at King Khalid University Hospital, Riyadh. ESBLs were confirmed using Etest strips of cefotaxime/cefotaxime + clavulanic acid, ceftazidime/ceftazidime + clavulanic acid, and cefepime/cefepime + clavulanate.

Results: Out of 17,105 samples, 1,076 (6.3%) ESBL-producing isolates of *E. coli* (808) and *K. pneumoniae* (268) were confirmed. Among these, 680 (63.2%) isolates were found in urine samples, followed by 287 (26.7%) in superficial swabs, deep wounds swabs, tissues and sterile body fluids, 71 (6.6%) in respiratory, and 38 (3.5%) in blood samples. The overall frequency rates of ESBL *E. coli* and *K. pneumoniae* were 6.6% and 5.5%, respectively. The frequency of ESBL-producing *E. coli* and *K. pneumoniae* increased significantly during the study period. *E. coli* resistance against cotrimoxazole was 71.1%, followed by ciprofloxacin (68.2%) and gentamicin (47%). Similarly, 62.7% of *K. pneumoniae* isolates were resistant to gentamicin, 59.5% to cotrimoxazole, and 49.8% to ciprofloxacin. There was no statistically significant change in antimicrobial resistance over the study period.

Conclusions: Although the frequency rates of ESBL-producing *E. coli* and *K. pneumoniae* increased, no change in the anti-microbial susceptibility was observed over the study period.

Key words: *Escherichia coli*; *Klebsiella pneumoniae*; extended-spectrum beta-lactamases; susceptibility; antibiotics.

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Introduction

Extended-spectrum β-lactamases (ESBLs) are known for their ability to hydrolyze β-lactam antibiotics such as penicillins, cephalosporins and monobactams, resulting in antimicrobial therapy failure [1]. These enzymes are inhibited by β-lactamase inhibitors such as clavulanic acid [2]. Strains producing ESBLs have emerged in Enterobacteriaceae, particularly among *E. coli* and *K. pneumoniae* [3]. A single organism may harbor multiple ESBLs such as the serine cephalosporinases and AmpC. A number of ESBL enzymes have been discovered recently in *E. coli* and *K. pneumoniae* [4]. Hospital-acquired infections due to ESBL-producing organisms are common, and several nosocomial outbreaks have been reported among patients in intensive care facilities [5]. Enterobacteriaceae, especially *E. coli*-producing β-lactamases, such as CTX-M enzymes, have been reported in the recent past as a common cause of urinary tract infections in community-based studies [6,7]. The incidence and pattern of antimicrobial resistance of ESBL-producing microorganisms such as *E. coli* and *K. pneumoniae* tend to exhibit regional variations [7]. Within the Kingdom of Saudi Arabia, the frequency of ESBL-producing *E. coli* and *K. pneumoniae* ranging between

4.8% and 15.8% has also been documented in different regions in the Kingdom [8-11]. These findings are consistent with a previously reported study describing considerable variations in the occurrence of ESBLs not only within countries but also from one hospital to another [12]. The aim of this study was to determine the frequency and antimicrobial resistance patterns among clinical isolates of ESBL-producing *E. coli* and *K. pneumoniae* from samples collected from hospitalized patients and those attending the outpatient clinics at the King Khalid University Hospital.

Methodology

Study design and study setting

The study was performed prospectively over a period of five years, from September 2006 to December 2010, at King Khalid University Hospital (KKUH), affiliated with King Saud University, a 950-bed teaching hospital serving as a primary, secondary, and tertiary referral center for more than one million Riyadh inhabitants.

Bacterial isolates

Consecutive non-duplicate *K. pneumoniae* and *E. coli* isolates recovered from clinical specimens from patients either admitted to different wards or attending the outpatient clinics at KKUH were included.All inoculation processes of the laboratory sample were performed under aseptic techniques by the technical personnel, who wore gloves and gowns in the biosafety cabinet. Sterile normal saline was used when needed, and the quality control policy was applied in sample processing at the medical microbiology laboratory of KKUH.

The automated MicroScan WalkAway-96 System was used to perform the identification and antimicrobial susceptibility tests using Negative Combo 30 B1017-302 and Negative Combo 34 B1017-305 panels (Dade Behring, Sacramento, CA, USA).

Double disk potentiation (DDP) test

Mueller-Hinton agar plates (MH II agar BBL, Becton Dickinson and Company, Cockeysville, Maryland, USA) were inoculated with the test organisms grown overnight on blood agar plates for the DDP method. Cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), and aztreonam (30 µg) disks were placed 30 mm (center to center) from the amoxicillin-clavulanate (20 µg and 10 µg) disk as recommended by Jarlier *et al*. [13]. After overnight incubation in air at 35°C, presumptive evidence for the presence of an ESBL was indicated by enhancement of the zone of inhibition of the ESBL antibiotic adjacent to the amoxicillin-clavulanate disk. This was recorded as DDP test positive.

Extended-spectrum beta-lactamase Etests (AB Biodisk, Solna, Sweden) were performed as confirmatory tests. Cefotaxime (CT) with a gradient concentration of 0.25–16 µg/mL and ceftazidime (TZ) with a gradient concentration 0.5–32 µg/mL was at one end; cefotaxime plus clavulanic acid (CTL) (0.016–1 µg/mL + 4 µg/mL) or ceftazidime with clavulanic acid (TZL) (0.064–4 µg/mL + 4 µg/mL) was at the other end. All antibiotics were used in accordance with the protocols from the manufacturer (AB Biodisk, Solna, Sweden). Results were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) [14]. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as control strains for all phenotypic ESBL testing.

This study was reviewed and approved by Institutional Review Board (IRB) at KKUH.

Statistical analysis

Statistical analysis was performed by the Chi-square test using SPSS version 19.0 statistical software (SPSS Inc. Wacker Drive, Chicago, IL USA); a pvalue of either equal to or less than 0.05 was considered statistically significant.

Results

The demographic information of patients with ESBL-producing *E. coli* and *K. pneumoniae* is shown in Table 1. There was a slight female preponderance in the outpatient group, and the majority of the hospitalized patients were older than 18 years of age. During the study period, a total of 17,105 strains of Enterobacteriaceae were isolated, including 12,224 strains of *E. coli* and 4,881 strains of *K. pneumoniae*. Among these, there were 1,076 (6.3%) ESBL isolates over the five-year study period, including 808 (75%) *E. coli* and 268 (25%) *K. pneumoniae*. The percentage of *E. coli* was consistently higher than that of *K. pneumoniae* in all types of specimens, with statistically significant differences among the respiratory and urine samples ($p = 0.02$ and $p = 0.003$, respectively).

Table 2 shows data about the occurrence of *K. pneumoniae* and *E. coli* in the patient samples. *K. pneumoniae* was most frequently isolated from the "others" category of samples, which comprised samples collected from superficial swabs, deep wound swabs, tissues, and sterile body fluids (7.4%),

followed by respiratory samples (5.9%). *E.coli* was most frequently found in respiratory samples (10.2%), followed by the "others" group, which comprised samples from various body sites (8.5%). Together, the highest occurrence of both the organisms was found in samples from various body sites (8%), followed by respiratory samples (7.09%).

The overall frequency rates of ESBLs among *E. coli* and *K. pneumoniae* were 6.6% and 5.5%, respectively. Table 3 describes the yearly frequency rates of ESBL-producing *E. coli* and *K. pneumoniae* between 2006 and 2010. A sharp increase in the frequency of *E. coli*, from 3.4% to 6.9%, was observed between 2006 and 2007 (p = 0.007). Between 2007 and 2008, the frequency rate of *E. coli* dropped from 6.9% to 4.9%, which was not statistically significant. No change between 2008 and 2009 (4.2%) was observed, and a significant increase in the frequency rate of *E. coli*, from 4.2% in 2009 to 6.3% in 2010, was observed (p = 0.04). Comparative analysis revealed that the frequency rate of *E. coli* in 2006 was significantly lower than in 2010 (p = 0.005). The frequency of ESBL *K. pneumoniae* isolates exhibited an increasing trend until 2008 and remained stable until 2010. There was a sharp increase in the frequency rate of *K. pneumoniae* between 2006 and 2007, from 3.3% to 6.3%, respectively. In 2008, the frequency of *K. pneumoniae* increased significantly to 8.1%, which was statistically higher than in the previous year (p = 0.01), and in the following years, the frequency rates of K. *pneumoniae* were 7.5% in 2009 and 7.9% in 2010 with no statistical difference. Comparison of the frequency rate of *K. pneumoniae* in 2006 with the frequency of *K. pneumoniae* in 2010 revealed a significant increase in 2010 (p = 0.0002).

Table 4 describes the antibiograms and frequencies of antimicrobial resistance of *K. pneumoniae* and *E. coli* during the study period. No significant difference in antimicrobial resistance between ESBL-producing *K. pneumoniae* and *E. coli* was observed among samples collected from different body sites. Antibiograms of 808 isolates of ESBL-producing *E. coli* from various body sites revealed a high percentage of resistance against cotrimoxazole and ciprofloxacin among blood isolates (91.67% and 79.17%, respectively), whereas the highest percentage of resistance to gentamicin (64.29%) was observed in respiratory isolates.

Table 1. Demographic information of patients infected with extended-spectrum beta lactamases, *E. coli* and *K. pneumoniae*

Site	*E. coli*	*K. pneumoniae*	P value	Total number of ESBLs/site
Respiratory	28/274 (10.2%)	43/727 (5.9%)	0.0259*	71/1,001 (7.09%)
Blood	25/382 (6.5%)	13/339 (3.8%)	0.1449	38/721 (5.3%)
Urine	570/9,409 (6.1%)	110/2,435 (4.5%)	0.0030*	680/11,844 (5.7%)
Others[#]	185/2,159 (8.5%)	102/1,380 (7.4%)	0.2677	287/3,539 (8%)

Table 2. Comparison of proportions of *E.* coli and *K. pneumoniae* among clinical samples
[#]Others: superficial swabs, deep wound swabs, tissues, and sterile body fluids; [*]Significant p value

	Hospitalized patient No. (%)	Outpatient No. (%)	Total (%)
Escherichia coli	460 (42.75%)	348 (32.34%)	808 (75.1)
Klebsiella pneumoniae	200 (18.58%)	68 (6.31%)	268 (24.9)
Gender			
Female	342 (31.78%)	286 (26.57%)	628 (58.4)
Male	318 (29.55%)	130 (12.08%)	448 (41.6)
Age			
> 18	505 (46.93%)	287 (26.67%)	793 (73.7)
≤ 18	155 (14.40%)	129 (11.98%)	283 (26.3)
Total	660 (61.3%)	416 (38.7)	1,076 (100)

Table 3. Comparison of the yearly frequency of ESBLs producing *E. coli* and *K. pneumoniae*

Year	*E. coli* frequency	*K. pneumoniae* frequency	Year of comparison	P value for *E. coli*	P value for *K. pneumoniae*
2006	3.3	3.4	2006-2007	0.0079[*]	0.0014[*]
2007	6.3	6.9	2007-2008	0.0177[*]	0.0710
2008	8.1	4.9	2008-2009	0.0177[*]	0.5049
2009	7.5	4.2	2009-2010	0.6286	0.0455[*]
2010	7.9	6.3	2006-2010	0.0002[*]	0.0052[*]

[*]Significant p value

Table 4. Percentage of resistance against different antibiotics in *K. pneumoniae* and *E. coli* ESBL isolates from various body sites

Year	Ciprofloxacin	Co-trimoxazole	Gentamicin	Amikacin	Nitrofurantoin
K. pneumoniae					
2006	12/18 (66.67)	13/18 (72.22)	11/19 (57.89)	0/9 (0)	2/5 (40)
2007	27/59 (45.76)	35/60 (58.33)	40/62 (64.51)	4/36 (11.11)	3/8 (37.5)
2008	26/57 (45.61)	26/57 (45.61)	31/58 (53.44)	2/20 (10)	1/4 (25)
2009	29/58 (50)	29/58 (50)	39/60 (65)	1/29 (3.44)	1/3 (33.33)
2010	28/53 (52.83)	44/54 (81.48)	37/53 (69.81)	2/26 (7.69)	5/18 (27.77)
Overall resistance	122/245(49.79)	147/247 (59.51)	158/252 (62.69)	9/120 (7.5)	12/38 (31.57)
E. coli					
2006	35/44 (79.55)	25/31 (80.64)	28/47 (59.57)	2/44 (4.54)	2/28 (7.14)
2007	96/140 (68.57)	87/113 (76.99)	86/152 (56.57)	11/152 (7.23)	6/102 (5.88)
2008	113/169 (63.13)	90/139 (64.74)	85/189 (44.97)	5/184 (2.71)	3/120 (2.5)
2009	129/194 (66.49)	102/143 (71.32)	87/199 (43.71)	5/196 (2.55)	5/136 (3.67)
2010	130/181 (71.82)	126/179 (70.39)	75/181 (41.43)	6/180 (3.33)	15/131 (11.45)
Overall resistance	503/738 (68.15)	430/605 (71.07)	361/768 (47)	29/756 (3.83)	31/517 (5.99)

*Others: superficial swabs, deep wound swabs, tissues, and sterile body fluids

Table 5. Consecutive five-year percent resistance of *K. pneumoniae* and *E coli* against various antibiotics

	Ciprofloxacin	Co-trimoxazole	Gentamicin	Amikacin	Nitrofurantoin
K. pneumoniae					
Blood	4/12 (33.33)	9/12 (75)	6/12 (50)	0/7 (0)	Not tested
Respiratory	23/43 (53.49)	25/41 (60.98)	28/41 (68.29)	2/22 (9.09)	Not tested
Urine	51/97 (52.58)	74/100 (74)	66/103 (64.08)	3/49 6.12)	11/32 (34.38)
Others*	44/95 (46.32)	64/95 (67.37)	58/96 (60.42)	4/42 (9.52)	Not tested
E. coli					
Blood	19/24 (79.17)	22/24 (91.67)	12/24 (50)	1/24 (4.17)	Not tested
Respiratory	21/28 (75)	21/28 (75)	18/28 (64.29)	0/28 (0)	Not tested
Urine	346/512 (67.58)	275/381 (72.18)	243/542 (44.83)	23/535 (4.30)	28/518 (5.41)
Others*	117/174 (67.24)	112/172 (65.12)	88/174 (50.57)	5/169 (2.96)	Not tested

Table 6. Comparison of resistance rates in *E. coli* and *K. pneumoniae* between hospitalized patients and outpatients

Organism and antibiotic	Hospitalized patients No. (%)	Outpatients No. (%)	P value
E. coli			
Ciprofloxacin	310/448 (69.2)	193/290 (66.55)	0.5004
Co-trimoxazole	280/397 (70.53)	150/208 (72.12)	0.7525
Gentamicin	237/438 (54.11)	124/320 (38.75)	< 0.0001
Amikacin	18/439 (4.1)	11/315 (3.5)	0.8122
Nitrofurantoin	17/250 (6.80)	14/275 (5.1)	0.5190
K. pneumoniae			
Ciprofloxacin	94/191 (49.21)	28/54 (51.85)	0.8505
Co-trimoxazole	128/189 (67.72)	44/59 (74.58)	0.4031
Gentamicin	122/191(63.87)	36/61 (59.02)	0.5962
Amikacin	6/90 (6.67)	3/29 (10.34)	0.8055
Nitrofurantoin	6/20 (30)	6/16 (37.50)	0.9056

Only 5.4% of the urine isolates were resistant to nitrofurantoin.Similarly, antibiograms of 268 isolates of ESBL-producing *K. pneumoniae* from various body sites tested in the study revealed that the highest percentage of resistance was against cotrimoxazole (75%), followed by gentamicin (68.29%) and ciprofloxacin (53.49%).

Table 5 shows yearly data of antibiotic resistance for both ESBL-producing *E. coli* and *K. pneumoniae*. Although the percentage of ESBL-producing *K. pneumoniae* resistant to cotrimoxazole and gentamicin increased between 2008 and 2010, this change was not statistically significant. No statistically significant differences in the resistance rates were detected for other antibiotics during the study period.

Table 6 compares the antibiotic resistance of ESBL-producing *E. coli* and *K. pneumoniae* isolated from hospitalized patients and from outpatients. Among all the non-β-lactam agents tested, a higher percentage of gentamicin resistance in ESBL-producing *E. coli* (54.11%) was found in samples from hospitalized patients compared to the percentage of gentamicin resistance in ESBL-producing *E. coli* (38.75%) present in samples collected from outpatients ($p< 0.0001$). There was no significant difference in antibiotic susceptibility of *K. pneumoniae* and *E. coli* against other antibiotics tested.

Discussion

This study reports the overall frequencies of ESBLs among *E. coli* and *K. pneumoniae* as 6.6% and 5.5%, respectively. Antibiotic resistance is a major public health concern worldwide. Varying frequencies of ESBLs among Enterobacteriaceae have been reported from many parts of the world [15-17]. Whereas a strikingly high frequency rate of ESBLs (44.9%) has been reported from Latin America [18], frequency rates ranging between as high as 27.4% in Greece and 15.5% in Portugal to as low as 2.6% in Germany and 2% in the Netherlands have been reported [19]. In the Gulf region, comparatively higher frequency rates of ESBLs – 31.7%, 22.6% and 41% – have been reported from Kuwait, Bahrain and United Arab Emirates, respectively [20-22]. Frequency rates of ESBLs among Enterobacteriaceae varying between 4.8% and 15.8% have been reported from the Kingdom of Saudi Arabia [8,11]. The eastern region in the Kingdom was found to have the lowest frequency rates [8], and the highest frequency of ESBLs was observed in the central region [11], pointing to the regional variation in the frequency rates within the country. Both studies involved a relatively smaller number of isolates over a shorter duration compared to the present study. Our study comprised 17,105 isolates of *E. coli* and *K. pneumoniae*,of which 1,076 (6.3%) were found to be ESBL producers. To our knowledge, this is the first study conducted in the Kingdom of Saudi Arabia comprising over a one thousand isolates investigating both the frequency and the susceptibility patterns of ESBL-producing Enterobacteriaceae.

The yearly frequency rates of *E. coli* and *K. pneumoniae* in the present study exhibited an upward trend and were significantly higher in 2010 compared with the frequency rates in 2006. In Ireland, the European Antimicrobial Resistance Surveillance System (EARSS) reported the most comprehensive data on ESBL prevalence in blood samples, describing an increase in the prevalence of ESBL among *E. coli* from 1.2% to 5% between 2002 and 2008 [23]. In a study from four hospitals performed over a period of four-and-a-half years, the prevalence of ESBL-producing *E. coli* from urine and blood cultures was found to have increased significantly over the study period [24]. Although ESBL-related prevalence studies are traditionally associated with healthcare settings, the increasing number of reports documenting the isolation of ESBLs in community settings also indicates increased prevalence of ESBLs [25]. The findings of the present study describing a significantly higher frequency of ESBLs over five years may not be applicable to the whole community, as the study focused on a single hospital's experience.

The frequency of ESBLs in *E. coli* and *K. pneumoniae* was lower than that reported in previous studies from Riyadh and Al-Khobar [26,27]. A study from Riyadh reported that a high percentage (55%) of *K. pneumoniae* was EBSL positive [26], and study from Al-Khobar revealed that 10.3% of *E. coli* and 12.2% of *K. pneumoniae* were ESBL positive [27]. This variation may be due to the difference in the number of isolates examined in these studies, as well as the setting – whether a tertiary care hospital or a community-based laboratory. The stable rate of ESBLs in our hospital reflects judicious use of antibiotics and effective implementation of infection control measures to control the spread of these strains.

In our study, none of the strains isolated were resistant to carbapenems. The efficacy of these anti-microbial agents has been attributed to their stability against beta-lactamases produced by ESBL bacteria; carbapenems therefore remain the treatment of choice for treating infections caused by ESBLs. Although the finding lacked statistical significance, the percentage

of ESBL-producing *E. coli* isolates resistant to amikacin, gentamicin, nitrofurantoin, and ciprofloxacin was higher in hospitalized patients compared with outpatients treated in the primary care setting. However, the percentage of isolates resistant to cotrimoxazole was higher in outpatients, possibly due to the increased use of this drug in the outpatient setting. Among *K. pneumoniae*, however, the percentage of isolates resistant to amikacin, nitrofurantoin, co-trimoxazole, and ciprofloxacin was higher in outpatients. A statistically significant difference in percentage of resistance was only detected for gentamicin, where the percentage of resistance among the hospitalized patients was higher than in the outpatients. This significant difference may have been due to the fact that gentamicin is used only in hospital settings. Moreover, a study from Norway investigating aminoglycoside resistance among *E.coli* and *Klebsiella* spp. isolates implicated the presence of aminoglycoside-modifying enzymes AAC(3)-II and AAC(6')-Ib in these bacteria in the acquisition of aminoglycoside resistance [28]. In addition, the emergence of CTX-M in the community, especially in urinary tract infections, may also have contributed to a higher antimicrobial resistance.

The higher rate of resistance against amikacin, nitrofurantoin, co-trimoxazole, and ciprofloxacin in the community is hard to explain, but the unwarranted use of antibiotics in the community may lead to organisms acquiring resistance. Several studies have documented a positive relationship between the use of third-generation cephalosporins, other β-lactams or fluoroquinolones and the acquisition of antimicrobial resistance by ESBL-producing organisms [29,30]. The high level of resistance to non-β-lactam classes of antibiotics observed in the present study is in agreement with previous reports implicating plasmid-mediated enzyme transfer between bacterial species by acquisition of genetic material related to antibiotic resistance [31]. Besides carbapenems, amikacin also exhibited potent antibacterial activity, but its use in clinical practice is limited because of its renal toxicity. Nitrofurantoin also performed better against ESBL-producing *E. coli* than against *K. pneumoniae*; this observation was consistent with a similar report from Spain [32]. Better performance of nitrofurantoin, particularly against *E. coli* compared to *K. pneumoniae*, has been reported in two separate studies from Iran and India; these studies, however, did not specify the ESBL status of the isolates [33,34]. The frequency of ESBL-producing *E. coli* was higher than that of *K. pneumoniae* among all clinical specimens included in the present study. This observation was consistent with a number of studies implicating Enterobacteriaceae – particularly *E. coli* – in urinary tract infections, which are believed to be the most common infections all over the world among hospitalized patients, including in the Kingdom of Saudi Arabia [8,10,21,22,27,35].

This study was limited by a lack of access to the clinical data. The findings of the present study, however, indicate that the frequency of ESBLs increased over the study period, thus emphasizing the need for introducing containment measures. Whereas no change was observed in the antibiotic susceptibility over the study period, carbapenems along with amikacin remain the drugs of choice to treat infections caused by ESBL-producing organisms. Both these classes of drugs need to be used judiciously to prevent the emerging resistance. Continued surveillance, appropriate use of antibiotics, and implementation of strict infection control measures are recommended to decrease ESBL frequency. Further studies involving molecular characterization of ESBLs are also recommended.

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Authors' contributions

Ali Somily was involved in study design, data analysis, literature review, and manuscript preparation. Hanan Habib was involved in study design, data analysis, and manuscript preparation. Muhammad Absar was involved in data collection, data analysis, and literature review. Muhammad Arshad was involved in data collection, data analysis, and literature review. Kutubu Manneh was involved in data collection, data analysis, and literature review. Sarah Al Subaie was involved in study design, data analysis, and manuscript preparation. Mogil Al Hedaithy was involved in study design, data analysis, and manuscript preparation. Samina Sayyed was involved in study design, data collection, data analysis, and literature review. Zahid Shakoor was involved in study design, data analysis, literature review, and manuscript preparation. Thomas Murray was involved in study design, data analysis, literature review, and manuscript preparation.

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