Letter to the Editor

ESBL-producing *E. coli* and *K. pneumoniae* in Al-Ahsa, Saudi Arabia: antibiotic susceptibility and prevalence of bla_{SHV} and bla_{TEM}

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Introduction

Antibiotic resistance is a serious problem in clinical medicine [1]. Production of inactivating enzymes is one of the most common mechanisms by which different microorganisms can acquire resistance against several antimicrobial agents, especially β-lactams. Extended spectrum β-lactamases (ESBLs) mediate resistance against different broad spectrum cephalosporins and monobactams [2].

There are different types of ESBLs, namely TEM, SHV, and CTX, but most of these enzymes are mutants of TEM1, TEM2, and SHV1 that are caused by point mutation of the original encoding genes [3]. These genes are carried on plasmids that may harbor other genes encoding resistance against different antimicrobial agents. These plasmids are easily transferred among different bacterial species, leading to widespread of multi-drug resistant bacteria [4]. *E. coli* and *Klebsiella* spp. are the most common producers of ESBLs and are responsible for many hospital- and community-acquired infections. Due to the extensive use of cephalosporins for treatment of different bacterial infections, the prevalence of ESBL-producing bacteria became significantly high worldwide [5].

In Saudi Arabia, limited data are available on the susceptibility patterns of ESBL-producing bacteria. The aim of the current study was to evaluate the susceptibility of ESBL-producing *K. pneumoniae* and *E. coli* clinical isolates to different antimicrobial agents and to estimate the prevalence of bla_{SHV} and bla_{TEM} in the tested isolates.

Methodology

A total of 97 non-duplicate ESBL-producing clinical isolates were collected from the Al-Ahsa region of Saudi Arabia. The isolates were identified by the Vitek 2 compact automated system (BioMerieux, Marcy L'Etoile, France) as *K. pneumoniae* (37 isolates) and *E. coli* (60 isolates) using GN cards.

The minimum inhibitory concentrations (MICs), resistance patterns, and preliminary phenotypic detection of ESBL production were determined using the Vitek 2 compact automated system using AST-N116 cards. Confirmation of ESBL production was done using the following Etest ESBL-strips (AB Biodisk, Solna, Sweden): Cefotaxime / cefotaxime plus clavulanic acid (CT/CTL), ceftazidime / ceftazidime plus clavulanic acid (TZ/TZL) and cefepime / cefepime plus clavulanic acid (PM/PML).

Polymerase chain reaction (PCR) amplification was used to detect the presence of genes encoding TEM and SHV enzymes as previously described [6]. For amplification of bla_{TEM}, the following primers were used: 5`-AGATCAGTTGGGTGCACGAG-3` and 5`-CAGTGCTGCAATGATACCGC-3`, while bla_{SHV} was detected using the following primers: 5`-CGCCTGTGTATTATCTCCC-3` and 5`-GGCGATTTGCTGATTTCGC-3`.

Table 1. Antimicrobial susceptibility patterns of ESBL-producing *E. coli* and *K. pneumoniae* isolates

Antimicrobial agents	*Escherichia coli* (n = 60)			*Klebsiella pneumoniae* (n = 37)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
	n (%)			*n* (%)		
Ampicillin	0 (0)	0 (0)	60 (100)	0 (0)	0 (0)	37 (100)
Ampicillin/Sulbactam	6 (10)	17 (28.3)	37 (61.7)	2 (5.4)	9 (24.3)	26 (70.3)
Piperacillin	0 (0)	0 (0)	60 (100)	0 (0)	0 (0)	37 (100)
Piperacillin/Tazobactam	48 (80)	6 (10)	6 (10)	17 (45.9)	4 (10.8)	16 (43.2)
Cefazolin	0 (0)	0 (0)	60 (100)	0 (0)	0 (0)	37 (100)
Cefuroxime	0 (0)	0 (0)	60 (100)	0 (0)	0 (0)	37 (100)
Cefuroxime Axetil	0 (0)	0 (0)	60 (100)	0 (0)	0 (0)	37 (100)
Cefoxitin	42 (70)	6 (10)	12 (20)	24 (64.9)	4 (10.8)	9 (24.3)
Cefpodoxime	0 (0)	0 (0)	60 (100)	0 (0)	0 (0)	37 (100)
Cefotaxime	11 (18.3)	8 (13.3)	41 (68.3)	7 (18.9)	5 (13.5)	25 (67.6)
Ceftazidime	21 (35)	34 (56.7)	5 (8.3)	5 (13.5)	15 (40.5)	17 (45.9)
Cefepime	46 (76.7)	2 (3.3)	12 (20)	24 (64.9)	5 (13.5)	8 (21.6)
Imipenem	60 (100)	0 (0)	0 (0)	37 (100)	0 (0)	0 (0)
Meropenem	60 (100)	0 (0)	0 (0)	37 (100)	0 (0)	0 (0)
Gentamicin	22 (36.7)	0 (0)	38 (63.3)	16 (43.2)	0 (0)	21 (56.8)
Tobramycin	15 (25)	13 (21.6)	32 (53.4)	7 (18.9)	14 (37.8)	16 (43.2)
Ciprofloxacin	13 (21.7)	1 (1.6)	46 (76.7)	6 (16.2)	5 (13.5)	26 (70.3)
Levofloxacin	14 (23.3)	0 (0)	46 (76.7)	8 (21.6)	8 (21.6)	21 (56.8)
Tigecycline	60 (100)	0 (0)	0 (0)	27 (73.0)	4 (10.8)	6 (16.2)
Sulphamethoxazole/Trimethoprim	13 (21.7)	0 (0)	47 (78.3)	7 (18.9)	0 (0)	30 (81.1)

Table 2. Prevalence of bla_{SHV} and bla_{TEM} in ESBL-producing *E. coli* and *K. pneumoniae* isolates

ESBL encoding gene	*Escherichia coli* No (%), 95% CI	*Klebsiella pneumoniae* No (%), 95% CI	P value *
bla_{SHV}	20 (33.3), 22.7-45.9	29 (78.4), 62.8-88.6	0.001
bla_{TEM}	44 (73.3), 58.7-80.8	16 (43.2), 28.7-59.1	0.005
bla_{SHV} and bla_{TEM}	11 (18.3), 10.6-29.9	8 (21.6), 11.4-37.2	0.894

* Z test for proportions (two ways)

Data analysis was done using SPSS version 16.0 (SPSS Inc, Chicago, USA). For categorical data, frequency, proportions, and percentage were used for reporting, and the Z test for proportions was used for comparison. Univariate analysis with estimation of odds ratio (95% confidence intervals) was also used for comparison. For continuous data mean, standard deviation and median were used for expression. A p-value of < 0.05 was used as the level of statistical significance.

Results and Discussion

The majority of the isolates were collected from urine specimens (35 *E. coli* and 14 *K. pneumoniae*) while only small numbers of isolates (two *E. coli* and one *K. pneumoniae*) were obtained from catheter tips. The rest of the isolates were collected from infected wounds (16 *E. coli* and 15 *K. pneumoniae*), from respiratory tracts, (four *E. coli* and six *K. pneumoniae*), and from blood (three *E. coli* and one *K. pneumoniae*). No significant difference was found in the prevalence of both pathogens recovered from different clinical specimens. Comparable patterns of microbial isolates were previously reported from different areas of Saudi Arabia [7,8].

The Vitek 2 compact system was used for detection of the ESBL-producing isolates and for confirmation of ESBLs production, three Etest ESBL-strips were used: CT/CTL, TZ/TZL, and PM/PML. The most sensitive strip was PM/PML, followed by CT/CTL; while TZ/TZL was the least sensitive one (data not shown). Similar results were previously published [9] showing that the PM/PML strip was the best configuration and the most suitable substitute for detection of ESBLs.

The antimicrobial susceptibility patterns of the tested isolates were depicted in Table 1. All isolates were resistant to ampicillin, piperacillin, cefazolin, cefuroxime, and cefpodoxime. Incorporation of tazobactam strongly affected the susceptibility of many tested isolates to piperacillin (67%) while sulbactam showed slight ability to reverse the

resistance of most isolates to ampicillin (8%). These results are consistent with recently published data [8].

The current study showed that carbapenems (imipenem and meropenem) were active against all tested isolates. Similar susceptibility to carbapenems (100%) was reported earlier [10,11]. In contrast, reduced susceptibility to imipenem and meropenem was found previously [12,13]. Nevertheless, carbapenems are still the drug of choice for the treatment of life-threatening enterobacterial infections caused by ESBL-producing pathogens [3]. Recently, many reports showing high levels of resistance to carbapenems were published [12,13]. Therefore, seeking out of alternatives that show *in vitro* activity against ESBL-producing pathogens (*e.g.*, fluoroquinolones, tigecycline, or antibiotic combinations) is important.

Tigecycline exhibited activity against all tested *E. coli* strains (MICs values were ≤ 4). Variable susceptibility was noted with *K. pneumoniae* isolates—susceptible (73%), intermediate (10.8%), and resistant (16.2%). Previously published reports showed that tigecycline exhibited excellent activity against ESBL-producing *E. coli* and *K. pneumoniae* [7,8]. Therefore, piperacillin-tazobactam and tigecycline hold promise to be alternatives that could limit the evolution and spread of carbapenem resistance.

Resistance to non β-lactam antimicrobial agents and third-generation cephalosporins is increasing globally. In this study, a high resistance rate was observed among tested isolates against sulphamethoxazole-trimethoprim (79%), ciprofloxacin (74%), levofloxacin (69%), gentamicin (61%), and tobramycin (49.5%). Co-resistance to fluoroquinolones, aminoglycosides, and sulphamethoxazole-trimethoprim was detected in sixteen *E. coli* and nine *K. pneumoniae* isolates (data not shown). In addition, four *Klebsiella pneumoniae* strains showed resistance to cefepime in addition to the three previously mention antimicrobial groups. Variable levels of resistance were reported in different local and regional studies [7,8,11,13]. This variation in the resistance/susceptibility patterns may be due to the types of antimicrobial agents commonly used in certain areas and the rate at which antibiotics are prescribed for treatment of various infectious diseases. Therefore, clinicians should be familiar with the antimicrobial stewardship programs to promote the optimum usage of antimicrobial agents (type, dose, duration, and route of administration) [14].

The prevalence of bla_{SHV} and bla_{TEM} in the tested isolates is summarized in Table 2. Bla_{SHV} was significantly dominant in *K. pneumoniae* isolates (78.4%). This result agreed with recently published data from Saudi Arabia [8,15]. On the other hand, bla_{TEM} was found to be highly prevalent in *E. coli* isolates (73.3%) in comparison with *K. pneumoniae* (43.2%). The prevalence of bla_{TEM} in Enterobacteriaceae differs across regions in Saudi Arabia [8,15]. Both bla_{SHV} and bla_{TEM} were coexistent in eleven (18%) *E. coli* and eight (22%) *K. pneumoniae* isolates, which was statistically insignificant. The detected bla_{SHV} and bla_{TEM} should be sequenced to explore the most prevalent type of β-lactamase genes in tested clinical isolates.

Conclusion

Because hospital settings are hotspots for the transmission of antibiotic resistance genes, a strict hospital infection control policy should be implemented; regular surveillance of microbial resistance is crucially needed. To ensure the judicious use of antibiotics, the local and regional data of resistance/susceptibility patterns should be available to clinicians, and effective local antibiotic policies should be applied. To limit the spread of multi-drug resistant pathogens, clinicians should test for ESBL-producing microorganisms along with the routine antimicrobial sensitivity testing they perform.

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