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The antimicrobial susceptibility profile of ESKAPE pathogens from urinary tract infections in a referral laboratory, Northeast Iran

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ABSTRACT

Objective: To assess the antimicrobial susceptibility pattern of ESKAPE pathogens from Neyshabur, Iran during 2013–2015.

Methods: A total of 345 isolates including 62 *Staphylococcus aureus* (*S. aureus*), 38 *Enterobacter* spp. (including 14 *Enterobacter agglomerans*, 6 *Enterobacter aerogenes* and other 18 *Enterobacter* spp.), 123 *Enterococcus faecium*, 78 *Klebsiella pneumonia*, 10 *Pseudomonas aeruginosa* and 34 *Acinetobacter baumannii* were isolated. The antimicrobial susceptibility pattern of isolates was conducted with Kirby Bauer method. Data were analyzed with SPSS 20.0 software using *F*- and *t*-tests.

Results: Among *S. aureus* isolates, the highest resistance was observed against nalidixic acid (81.35%) and cefixime (74.50%). Thirty-three (53.22%) *S. aureus* isolates were cefoxitin resistant (methicillin-resistant *S. aureus*). The majority of *Enterobacter* species was resistant to amikacin (100.00%) and cephalotin (66.60%). Most *Enterococcus faecium* isolates were resistant to nalidixic acid (89.43%) and amikacin (83.33%), but vancomycin-resistant enterococci isolates were not detected. Moreover, among *Klebsiella pneumonia*, the highest resistance was observed to nalidixic acid (20.98%) and cotrimoxazole (28.39%). Furthermore, all *Pseudomonas aeruginosa* isolates were resistant to cefotaxime (100.00%) and majority to nitroforantoin (88.80%). *Acinetobacter baumannii* isolates showed the highest and the lowest resistance to cefotaxime (100.00%) and cefixime (88.71%), respectively.

Conclusions: The prevalence of ESKAPE pathogens from northeast region was low, but majority of them exhibited high rate of antibiotic resistance to common used antimicrobial agents.

1. Introduction

ESKAPE pathogens, isolated from hospital and community settings, have been recently categorized because of new paradigms in pathogenesis and transmission and overcoming to drugs effectiveness. It has been revealed that ESKAPE pathogens are involved in nearly 41% of infections in patients in intensive care units[1]. Resistance levels are substantial in these pathogens, ranging from resistance that approximately completely excludes an antibiotic from empirical therapeutic insights [*e.g. Enterococcus faecium* (*E. faecium*) with resistance to ampicillin or vancomycin] to resistance that shows the potential to change choices of both empirical and definitive antimicrobial therapies [*e.g. Acinetobacter baumannii* (*A. baumannii*) or *Pseudomonas aeruginosa* (*P. aeruginosa*) with resistance to carbapenems][2,3]. However, the rates of resistance vary in different areas. *Staphylococcus aureus* (*S. aureus*) with resistance to methicillin [methicillin-resistant *S. aureus* (MRSA)] and intermediate level vancomycin (vancomycin-intermediate *S. aureus* strains) are examples of drug resistant *S. aureus* from hospital or community settings[4,5]. Emergence of extended spectrum beta-lactamases (ESBL) and carbapenemase enzymes among Gram-negative species have been developed worldwide[6,7]. *P. aeruginosa* has long been the "holy grail" target for antimicrobial development. The importance of *P. aeruginosa* in causing deaths of patients with febrile neutropenia and its intrinsic resistance to many early antimicrobial agents led to concerted efforts to find new antibiotics with activity against this species[8,9]. Until now,

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there has been no previous report of ESKAPE prevalence and their antimicrobial susceptibility profile collectively in one study in Iran. The aim of this study was to determine prevalence and antimicrobial susceptibility pattern of ESKAPE isolates from Neyshabur, Northeast Iran during 2013–2015.

2. Materials and methods

2.1. Study sample and design

During the years 2013–2015, a total of 1661 patients (age ranged from 7 months to 74 years old) with urinary tract complications were referred to the referral laboratory of Neyshabur. The midstream urine of them was collected and cultured. The bacterial species were identified by using conventional biochemical tests. Among them, 345 ESKAPE pathogens including 62 *S. aureus*, 18 *Enterobacter* spp., 14 *Enterobacter agglomerans* (*E. aglomerans*), 6 *Enterobacter aerogenes* (*E. aerogenes*), 123 *E. faecium*, 78 *Klebsiella pneumonia* (*K. pneumonia*), 10 *P. aeruginosa* and 34 *A. baumannii* were isolated.

2.2. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of isolates was conducted with Kirby Bauer method, and following Clinical and Laboratory Standards Institute advice, version 2013[10]. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 standard strains were used as quality control for the disks. The disks included: nalidixic acid, nitrofurantoin, chloramphenicol, ciprofloxacin, cefoxitin, ceftizoxime, ceftazidime, cotrimoxazol, gentamicin, cefalotin, amikacin, cefotaxime and erythromycin (Padtan Teb). Ceftazidime and cefoxitin resistance was considered as ESBL producers and MRSA strains, respectively.

2.3. Ethical approval

The study protocol was performed according to the Clinical And Laboratory Standards Institute declaration and approved by Academic Center for Education, Culture and Research. Informed written consent was obtained from Academic Center for Education, Culture and Research committee.

2.4. Data analysis

Data were analyzed with SPSS 20.0 software using *F*- and *t*-tests. Any $P < 0.05$ [95% confidence interval (*CI*)] was considered as significant difference.

3. Results

The prevalence of ESKAPE pathogens was 20.77%, including 3.73% *S. aureus*, 2.29% *Enterobacter* spp., 7.40% *E. faecium*, 4.69% *K. pneumonia*, 0.60% *P. aeruginosa* and 2.04% *A. baumannii*. The age average of patients was (33.10 ± 24.16) years. All these pathogens isolated from female patients were more significantly than those from male patients (Table 1). Other organisms other than ESKAPE cases included: 1180 *E. coli*, 5 *Citrobacter diversus*, 5 *Citrobacter freundii*, 1 *Streptococcus pyogenes*, 69 *Proteus mirabilis*, 1 *Proteus vulgaris*, 1 *Serratia marssecence*, 23 *Staphylococcus epidermidis* and 32 *Staphylococcus saprophyticus*. The number and percentage of ESKAPE isolates are depicted in Table 1. The antibiotic susceptibility profile of ESKAPE pathogens is exhibited in Tables 2 and 3. It was shown that 53.22% of *S. aureus* isolates were resistant to cefoxitin (indicating MRSA). Moreover, the rate of ceftazidime resistance among *Enterobacter* spp., *E. faecium*, *K. pneumonia*, *P. aeruginosa* and *A. baumannii* was 20.00%, 30.00%, 40.00%, and 81.00%, respectively. In addition, 426/1180 (36.10%) *E. coli*, 1 (20%) *Citrobacter diversus*, 3 (4.34%) *Proteus mirabilis* were ceftazidime resistant, but all *Serratia* spp. were susceptible to it.

Table 1
The distribution of ESKAPE isolates among female and male patients.

Genus	Species	Number	Male (%)	Female (%)	*P* value (95% *CI*)
Enterobacter	*Enterobacter* spp.	18	39.47	50.53	0.001 6
	E. aerogenes	6			
	E. agglomerans	14			
Entroccoci	*E. faecium*	123	16.26	82.74	< 0.000 1
Klebsiella	*K. pneumonia*	78	16.25	82.75	< 0.000 1
	Klebsiella ozaenae	2			
Pseudomonas	*P. aeruginosa*	10	30.00	70.00	0.000 8
Staphylococcus	*S. aureus*	62	9.67	90.23	< 0.000 1
Acinetobacter	*A. baumannii*	34	26.47	72.53	< 0.000 1

Table 2
The antibiotic resistance (%) profile of ESKAPE pathogens

Disks/isolates	*Enterobacter* spp.	*E. faecium*	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*A. baumannii*
Cefixime	30.30	56.38	0.00	85.71	74.50	88.71
Nalidixic acid	42.85	89.43	20.98	60.00	81.35	66.00
Nitrofurantoin	33.00	4.88	13.58	88.80	0.00	89.00
Chloramphenicol	16.66	15.25	19.60	10.00	34.00	10.00
Ciprofloxacin	16.20	12.39	8.64	10.00	9.30	40.00
Ceftizoxim	8.10	1.00	4.94	80.00	1.63	80.00
Cotrimoxazole	40.00	61.98	28.39	60.00	16.07	63.00
Gentamicin	21.20	83.00	12.85	12.50	2.17	16.50
Cefalotin	66.60	100.00	40.00	100.00	0.00	100.00
Amikacin	100.00	83.33	11.00	12.00	3.78	14.00
Cefotaxime	21.43	22.06	28.20	92.00	23.52	100.00
[a]Ceftazidime	20.00	–	30.00	40.00	-	81.00
Cefoxitin	80.00	60.00	90.00	100.00	53.22[b]	93.00

[a]: Supposed possible ESBL producing isolates; [b]: MRSA strains.

Table 3
The sensitivity level (%) of ESKAPE isolates.

Disks/isolates	*Enterobacter* spp.	*E. faecium*	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*A. baumannii*
Cefixime	75.8	38.0	52.0	0.0	11.0	2.0
Nalidixic acid	79.3	12.0	64.0	2.0	6.0	2.0
Nitrofurantoin	40.0	64.0	65.0	1.0	61.0	0.0
Chloramphenicol	69.0	49.0	41.0	54.0	31.0	42.0
Ciprofloxacin	83.8	40.0	73.0	46.0	54.0	45.0
Ceftizoxim	91.9	93.0	71.0	4.0	45.0	3.0
Cotrimoxazole	60.0	42.0	58.0	20.0	47.0	20.0
Gentamicin	88.0	12.0	59.0	40.0	46.0	32.0
Cefalotin	20.0	0.0	3.0	0.0	90.0	0.0
Amikacin	0.0	10.5	34.0	36.0	38.0	27.0
Cefotaxime	70.0	52.0	28.0	0.0	12.0	0.0
Ceftazidime	71.0	–	50.0	12.0	–	2.0
Cefoxitin	10.0	40.0	4.0	2.0	30.0	0.0

4. Discussion

Knowledge of local antibiotic resistance trends among urinary isolates is crucial not only in guiding clinicians to prescribe appropriate antibiotics but also for observations based recommendations for empirical antibiotic treatment of urinary tract infection[11]. The current study assesses the antimicrobial resistance rates among ESKAPE isolates and possible detection of ESBL production and MRSA among urinary isolates in Neyshabur, Iran. In the current study, the age average of patients was (33.1 ± 24.16) years. Moreover, the age range of patients was 7 months to 74 years old. In this study, the history of patients regarding prior antibiotic consumption, hospitalization, catheter, smoking and contact with hospital settings was not elucidated. Thus we could not determine any relation between most of possible

risk factors and presence of ESKAPE isolates.

The prevalence of ESKAPE pathogens from midstream urine was 20.77%, including 3.73% *S. aureus*, 2.29% *Enterobacter* spp., 7.40% *E. faecium*, 4.69% *K. pneumonia*, 0.60% *P. aeruginosa* and 2.04% *A. baumannii*. In this study, *E. faecium* were the most common isolates from urinary tract infections. Enterococci have been reported from urinary tract infections and aminopenicillins may be used as a resort for vancomycin-resistant enterococci[12]. A study showed that the midstream urine of patients contained 1% *P. aeruginosa* and *K. pneumonia* and 18% enterococci, but no other ESKAPE isolates were determined[13]. It was shown that 53.22% of *S. aureus* isolates were resistant to cefoxitin (indicating MRSA). Sasirekha demonstrated that among 325 clinical isolates from urinary tract infection, the prevalence of MRSA was 27.5% and ESBL positive Gram-negative bacteria were 48.9%[14]. In this study, the rate of ceftazidime resistance among *Enterobacter* spp., *K. pneumonia*, *P. aeruginosa* and *A. baumannii* was 20.00%, 30.00%, 40.00% and 81.00%, respectively. It was proposed that ceftazidime resistance is a potential for ESBL production.

Antibiotic resistant pathogens have caused rising in morbidity and mortality and increase of economic costs and hospitalization. Vancomycin-resistant enterococci, MRSA and ESBL producer Gram-negative bacteria confer these conditions as reported previously[6,15-17].

Among the oral antibiotics, nitrofurantoin commonly prescribed for urinary tract infections in most of countries showed a high resistance rate against *A. baumannii* (89.00%) and *P. aeruginosa* (88.80%) in our study which highlighted an increased resistance among these organisms in this area, while it was more effective against *K. pneumonia* and *Enterobacter* spp., which are consistent with those of the previous studies[18-22]. A low degree of resistance to gentamicin and amikacin was observed for *K. pneumonia*, *P. aeruginosa*, *S. aureus*, and *A. baumannii*, thus may be helpful in combating severe infections. In our study, carbapenems were not evaluated.

The current study, which examines the prevalence of ESKAPE uropathogens and spread of drug resistance among them, is possibly first of its kind as it is not restricted to a specific pathogen and health care center. On the basis of these findings, it is suggested that urine culture and antimicrobial susceptibility testing for urinary tract infections. Continuous analysis of the antibiotic resistance profile acts as a guide in initiating the empirical treatment of urinary tract infections. Development of local surveillance programs is necessary to provide information which would then enable the development of specific urinary tract infection guidelines.

Conflict of interest statement

We declare that we have no conflict of interest.

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