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Research Article

Extended Spectrum Beta Lactamase Producing Escherichia Coli and Klebsiella Isolates in Patients with Urinary Tract Infection and Their Antibiotic Susceptibility Pattern in Babol, Northern Iran

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ABSTRACT

The major mechanism of resistance to antimicrobial agents especially between gram negative bacteria is the production of extended-spectrumbeta lactamase (ESBL) enzymes. The aim of this study was determine the prevalence and antibiotic susceptibility pattern of ESBL producing E.coliand Klebsiella isolates in patients with UTI.A total 4957 urine samples from patients referring to Yahyanejad Hospital,Babol,were collected from August 2016 to August 2017. The samples were evaluated for bacterial culture using conventional methods. ESBL production were determined by Double-Disk method. Antibiotic susceptibility test for ESBL producing organisms was done by disc diffusion method. A total of 352 samples showed growth of pathogens and among hospitalized patients, 40.94% of E.coli and 31.8% of Klebsiellawere ESBL producers and betweennon-hospitalized patients, the rate of ESBL productionwas (29.76%) and (12.5%), in E.coli and Klebsiella isolates, respectively.Percentage of susceptibility in E.coli and Klebciella isolates was (95.6% and 85.7%) to piperacillin-tazobactam, (93.3% and 75%) to meropenem, (93.8% and 70%) to amikacin, and (91.4% and 25%) to nitrofurantoin, respectively, While high percent of resistance in E.coli and Klebsiella isolates was found against cefotaxime (95.8% and 85.7%), ceftriaxone (91.7% and 66 .7%) and ceftazidime (73.3% and 66.7%), ciprofloxacin (7o.4% and 50%) respectively. With regard to low resistance to nitrofurantoin, this drug can be prescribed in uncomplicated UTI and duo to high rate of resistance to cephalosporins, these antimicrobial agents are not appropriate choices for treatment of complicated UTI and piperacillin-tazobactam, amikacin and meropenem are better choices for this goal.

Keywords: extended-spectrumbeta lactamase, urinary tract infections, E.coli, Klebsiella, antibiotic resistance

INTRODUCTION

One of the most common infections in participants of both inpatients and outpatients setting is urinary tract infection (UTIs)(1, 2). UTIs caused by multidrug-Resistancebacteria, are a serious global health concern duo to limited treatment options(3). So physicians need to be aware of epidemiological and clinical risk factors of these infections, such as prior history of UTI, recent antibiotic use, recent hospital stay, chronic medical conditions and age over 65 years(4). Beta lactam antibiotics are the most common antimicrobial agents that are prescribed by clinicians.The major mechanism of resistance to antimicrobial agents especiallybetweengram negative bacteria is the production of extended-spectrum beta lactamase (ESBL) enzymes(5). ESBLs can hydrolyze penicillins, advanced cephalosporins and monobactams. They are

plasmid mediated which can carry genes that encode resistant to other antimicrobial agents, such as aminoglycosides and quinolones(6). Therefore ESBL producer organisms became a serious problems in clinical practice and can lead to therapeutic failure(7). The most production of ESBL enzymes have been reported in E.coli and Klebsiella Pneumoniae(8).The incidence of infections caused by ESBL producing bacteria is increasing worldwide and have been associated with adverse clinical outcomes or even death. To avoid of such adverse events, an appropriate antimicrobial management will be needed(9).To achieve this goal, aware of local hospital based knowledge of the resistance pattern of the bacterial uropathogens is necessary(10).The aim of this study was to determine the prevalence and antimicrobial susceptibility pattern of ESBL producing E.coli and Klebsiella isolates in patients with UTI.

METHODS

This cross-sectional study was done in ShahidYahyanejad Hospital, Babol, north of Iran, over a period of 12 months,from August 2016 to August 2017. Urine samples from outpatients and inpatients were collected and cultured on blood agar and Mac Conkey agar mediums by the standard method. E.coli and Klebsiella isolates identified with biochemical tests in TSI (Triple Sugar Iron) agar medium.

Determination of the ESBL production was done by Double-Disc method. The bacterial suspension was prepared for dilution method with 0/5 Mcfarland standard and was cultured on Muller-Hinton agar plates. Four separate discs containing 30 µgr of ceftazidime and 30 µgr of cefotaxime alone and in combination with 10 µgr of clavulanic acid, were placed on the agar. After 24 h incubation at 37°C, an increase in the diameter of inhibition zone more than or equal to 5 mm for a combination disc versus the disc when tested alone, was considered to determine the presence of ESBL in the organisms.Klebsiella ATCC 700603 was used for quality control as recommended by CLSI (Clinical Laboratory Standard Institute).We developed a questionnaire about demographic and clinical information of patients, contained age, gender, co-morbidities, the hospital ward they were admitted (for inpatients), urethral catheter use for the past 1 year, recent urinary infection, recurrent UTI (more than 3 times), hospitalization for the past three months, antibiotic consumption in the preceding three months. After obtaining consentience, we filled the questionnaire for patients with ESBL-positive UTI.Antibiotic susceptibility pattern was determined by disc diffusion method on Muller-Hinton agar, according to CLSI guidelines(11). The antimicrobial agents that gram negative bacteria were tested against them were: amikacin(AMK 30 µgr), piperacillin-tazobactam (PTZ 100µgr), meropenem (MEM 10 µgr), ampicillin-sulbactam (SAM 20µgr), ceftazidime (CAZ 30 µgr), cefotaxime (CTX 30 µgr), ceftriaxone (CRO 30 µgr), ciprofloxacin (CIP 5µgr) and nitrofurantoin (NIT 30 µgr). All antibiotic discs were obtained from MAST chemical co,England.Quality control was tested by E.coli ATCC 25922 AS CLSI recommended. This study protocol was approved by ethics committee of Babol University of Medical sciences. All data were analyzed by SPSS software 16.

RESULTS

During 12 month, a total of 2085 and 2872 urine samples were collected from inpatients and outpatients,respectively. Table 1 shows the total number of positive culture and the sex distribution and overall prevalence of E.coli and Klebsiella isolates in hospitalized and non-hospitalized patients. As you see, in both groups prevalence of E.coli isolates were higher than Klebsiella isolates and the majority of the patients were female.

Among 127 E.coli and 22 Klebsiella isolated from inpatients, 52 (40.9%) and 7 (31.8%) were ESBL producers, respectively and among 84 E.coli and 24 Klebsiella isolated from outpatients, 25 (29.8%) and 3 (12.5%) were shown ESBL production. The rate of ESBL production in E.coli isolates was higher than Klebsiella isolates and also in hospitalized patients was more than non-hospitalized patients, as shown in figure 1.

Total Number	Hospitalized (%)	Non-hospitalized(%)
Positive urine culture	196(100)	156(100)
E.coli	127(64.79)	84(53.84)
Klebsiella	22(11.22)	24(15.38)
Female	128(65.3)	131(83.97)
Male	68(34.69)	25(16.02)

Table 1. Total distribution of sex and overall prevalence of E.coli and Klebsiella isolates in hospitalized and non-hospitalized patients

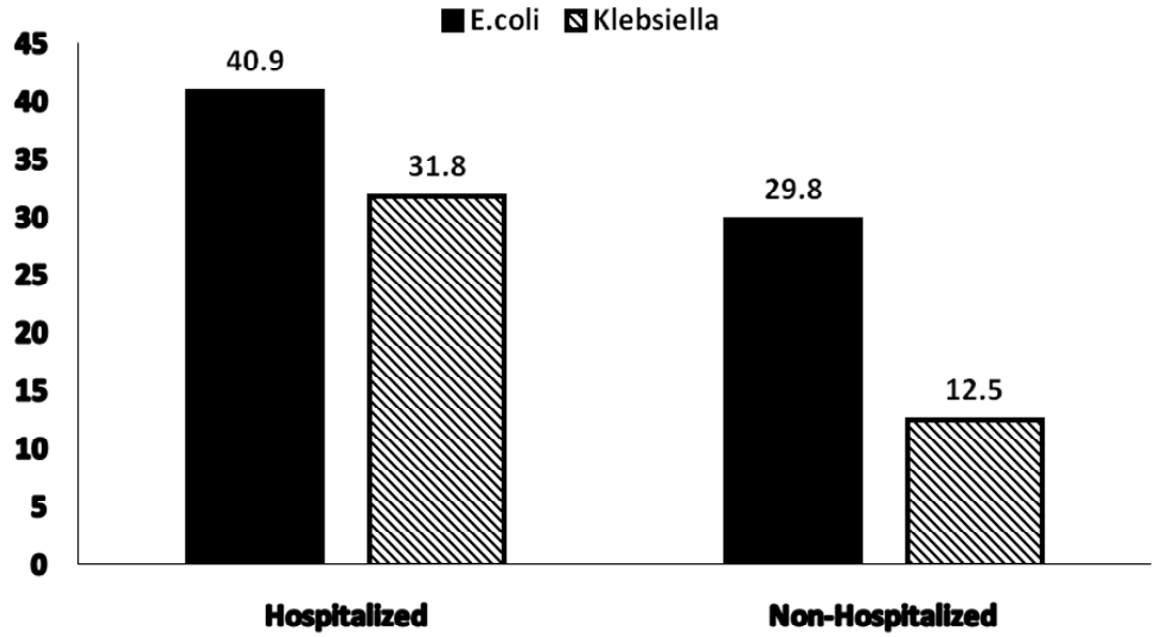

Figure 1. Prevalence of ESBLs in E.coli and Klebsiella isolates in hospitalized and non-hospitalized patients.

Characteristics of the patients (percent) with ESBL UTI is shown in Table 2. The age range of the patients was between 25-93 years old and most of the patients were in the age group of 60 years and older. The majority of the patients had history of antibiotic consumption. History of recent hospitalization was 30% and 60.4% in outpatients and inpatients, respectively. History of prior use of catheter was 45.3% in hospitalized patients while no history of urine catheterization was seen in non-hospitalized patients. History of prior UTI in both groups was high, 60% and 67.9% in outpatients and inpatients, respectively. In terms of underlying illnesses, in hospitalized patients, rate of heart disease was 56.6% and diabetes was seen in 24.6% of cases while in non-hospitalized patients, rate of diabetes was 40% and there were no cases of heart disease. Among hospital wards, 47.5% of inpatients were in emergency room, as shown in Table 3.

Characteristic	Hospitalized(%)	Non-Hospitalized(%)	Total(%)
Gender			
Female	76.3	89.3	80.5
Male	23.7	10.7	19.5
Age(years)			
20-39	12.1	32	18.1
40-59	22.4	32	25.3
≥60	65.5	36	56.6
Co-morbidities			

Diabetes	24.6	40	27
Heart disease	56.6	0	47.6
Chronic kidney disease	13.2	50	19
Renal stone	1.9	10	3.2
Prior(1 year)urinary catheter	45.3	0	38.1
Prior (1 year) UTI	67.9	60	66.7
More than three UTI in the preceding year	31.4	40	32.8
Prior (3 months)hospitalized	60.4	30	55.6
Prior (3 months) antibiotic exposure	71.7	50	63.3

Table 2. Characteristics of the patients with ESBL UTI

Hospital ward	Number of patients(%)
Emergency	28 (47.5)
Heart	13 (22)
Internal	6 (10.2)
Infection	4 (6.8)
Psychiatry	2 (3.4)
Surgery	1 (1.7)
midwifery	1 (1.7)
ICU	2 (3.4)

Table 3. Percentage of inpatients in hospital wards

Table 4 shows antibiotic susceptibility and resistance percentages of ESBL producing organisms in outpatients and inpatients. In both groups, the highest percent susceptibility was seen to piperacillin-tazobactam, 93.9% in hospitalized and 100% in non-hospitalized patients and the maximum resistance was seen against cefotaxime and ceftriaxone.

In both E.coli andKlebsiella isolates, piperacillin-tazobactam, amikacin and meropenem were the most effective antibiotics while resistance against cefotaxime, ceftriaxone and ceftazidime were markedly higher than other antimicrobial agents, as shown in figure 2.

Antibiotics	Hospitalized		Non-hospitalized		Total	
	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)
AK	88.5	11.5	95.7	4.3	90.7	9.3
PAZ	93.9	6.1	100	0	94.2	5.8
MEM	92	8	66.7	33.3	90.6	9.4
SAM	52.4	47.6	70.6	29.4	57.6	42.4
CRO	5.5	94.5	23.1	76.9	11.1	88.9
CTX	2.6	97.4	11.8	88.2	5.5	94.5
CAZ	27.3	72.7	27.3	72.7	27.3	72.7
CP	24.1	75.9	48.1	51.9	32.1	67.9
FM	86.4	13.6	82.4	17.6	84.6	15.4

Table 4. Resistanse (%) of ESBL producing organisms in outpatients and inpatients

R=Resistant; S=Susceptible; AK=Amicacin; PAZ=Piperacillin-tazobactam; MEM= Meropenem; SAM= Ampicillin-sulbactam; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CP= Ciprofloxacin; FM= Nitrofurantoin

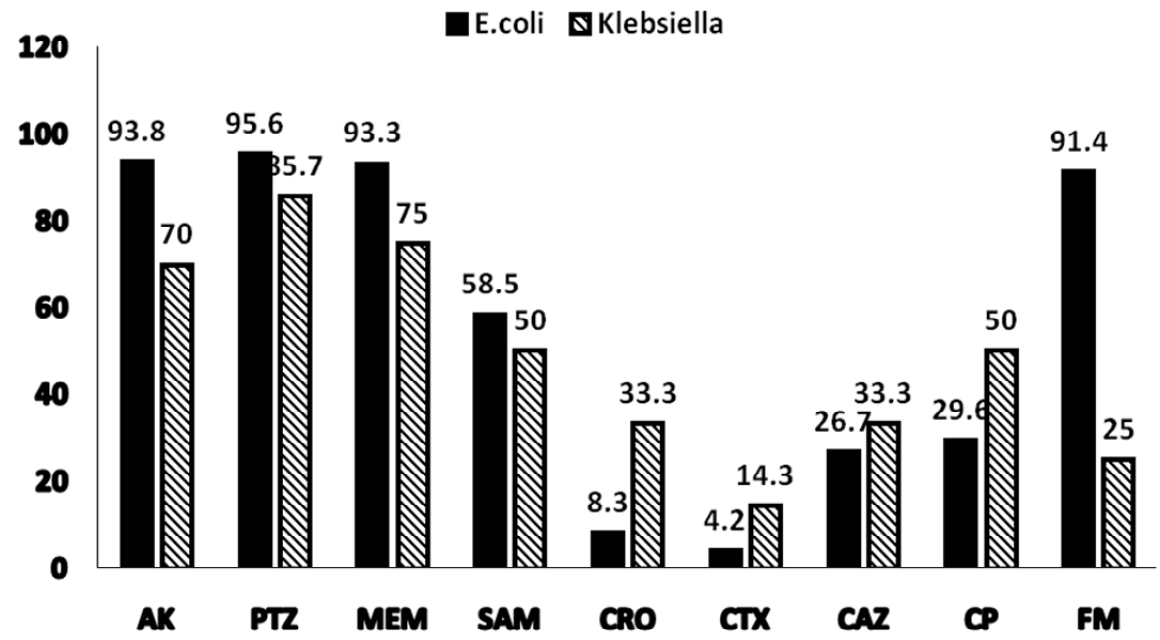

Figure 2. Percentages of antibiotic susceptibility in ESBL producing E.coli and Klebsiella isolates
Amicacin (AK);Piperacillin-tazobactam (PAZ); Meropenem (MEM); Ampicillin-sulbactam (SAM); Ceftriaxone (CRO); Cefotaxime (CTX); Ceftazidime (CAZ); Ciprofloxacin (CP); Nitrofurantoin (FM)

DISCUSSION

The relatively high prevalence of ESBL producing bacteria and high resistance to broad-spectrum antibiotics,is alarming. Prevalence of ESBL-positive organisms in different country and even in different treatment centers is vary(12). In our study, overall prevalence of ESBL production in E.coli isolates was 36.5% (40.99% in hospitalized and 29.76% in non-hospitalized) and in Klebsiella isolates was 21.7% (40.94% in hospitalized and 12.5% in non-hospitalized). In a study in Isfahan by Moayednia et al, prevalence of ESBL production in E.coli isolates was 40% in hospitalized and 25% in non-hospitalized, that was similar to our investigation, but in Klebsiella isolates, rate of ESBL production was 51.5% in the inpatients and 40% in the outpatients, that was higher than our reports (13).In another study in Thailand in 2011, ESBL production was detected in 17% of E.coli and 34.5% of Klebsiella isolates (14).Goncalves LF et al, in Brazil reported that among 324 E.coli isolates from outpatients, 23 (7.1%) were identified as ESBL producers and in the similar study in India, this rate was reported 34.42% (15, 16). Significant difference in the prevalence of ESBL production between E.coli and Klebsiella isolates in different parts of the world maybe duo to the use of different and non-standard laboratory methods in identifying ESBL producing bacteria, so to minimize these differences, fallow the international guidelines is recommended.

Awareness of physicians from patient`s risk factors for antibiotic resistance will help them to select appropriate empirical therapy(4). In the present investigation, majority (88%) of the ESBL producing isolates were E.coliand the remaining 12%, were belong to Klebsiella. Similarity to our study was reported in Seri Lanka in 2012: 86.2% of E.coli and 13.8% of Klebsiellaisolates were ESBL producers(17).

History of antibiotic use was 71.7% in hospitalized and 50% in non-hospitalized patients. In a study from Turkey was reported that 20% of outpatients with ESBL-positive UTI had prior use of antibiotics that this finding was low as compared to our study (18). Another study by Sibhghatulla Shaikh et al showed 38.32% of ESBL producing E.coli and

Klebsiella isolates in hospitalized patients had recent exposure to antibiotics(7). Frequent and uncontrolled use of antimicrobial agents and easy access to antibiotics without prescription in drug stores in our county maybe justifiable for the high percentage of multidrug resistance organisms in this region.

History of hospitalization in the inpatients of our investigation was 60.4%, this rate was 21.4% in Japan and 49.1% in Seri Lanka reports that were lower than the results of our study (17, 19). History of UTI and recurrent UTI in outpatients of our investigation were 60% and 40%, respectively, that was high as compared to the report of Turkey: 18% for UTI and 21% for recurrent UTI, also in the Turkish study, prior urinary catheter was seen in 13% of outpatients both there is no cases of catheterization in outpatients of our study (18).

Among co-morbidities, history of Diabetes was found in 24.6% of our hospitalized patients while rate of diabetes in India, Seri Lanka, Switzerland and Japan was 25.75%, 88.5%, 40% and 35.7%, respectively (7, 17, 19, 20). In our report, prior use of antibiotics and prior UTI, were among the most common risk factors for getting multidrug resistance UTIs.

Antibiotic resistance patterns are constantly changing and vary in different geographical areas, so regular surveillance of antibiotic susceptibility pattern is needed to set up safe and effective empirical therapy(21, 22). In the recent study, the highest percentage of susceptibility in ESBL producers, was found to piperacillin-tazobactam (PTZ), meropenem (MEM) and amikacin (AK), ranging from (93.3%- 95.6%) for E.coli and (70%- 85.7%) for Klebsiella isolates. Suseptibility to nitrofurantoin (FM), in E.coli isolates was 91.4%, but only 25% of Klebsiella isolates was sensitive to this antibiotic. Both hospitalized and non-hospitalized patients also had the highest sensitivity to PTZ, (93.9% and 100%), respectively and followed byMEM, AK and FM. These finding are consistent with the results reported by many studies, for example, in a study from Iran in 2012, E.coli isolates had 98% sensitivity to MEM, 95% to AK and 91% to FMand in another study in Isfahan, Iran, sensitivity to MEM and FM was high but contrary to our study, susceptibility to PTZ was 57% in E.coli and 38.8% in Klebsiella isolates(22, 23). In Indian report in 2014, susceptibility to MEM was 98.9% in E.coli and 97.3% in Klebsiella isolates, but sensitivity to AK was 31.19% and 40.54% in E.coli and Klebsiella isolates, respectively(7). Also FM is one of the oldest drugs for UTI treatment, the rate of susceptibility to this drug remains too low, maybe duo to limited use of FM as first line antibiotic for treatment of uncomplicated UTI in Iran.

Our ESBL-positive isolates showed high resistance against cefotaxime(CTX), ceftriaxone (CRO) and ceftazidime (CAZ), ranging from (73.3-95.8%) for E.coli and (66.7-85.7%) for Klebsiella isolates. Resistance to ciprofloxacin (CP) was found in 70.4% and 50% of E.coli and Klebsiella isolates, respectively. High prevalence of resistance to these antibiotics is probably the result of extensive use of them during the past decade. Similarity to our study, in Indian report in 2014, resistance to CRO, CTX, CAZ and CP was high, ranging from (56.99-79.73%) (7). In Isfahan in 2012, rate of resistance to CAZ and CTX especially between non-hospitalized patients was lower than our results, ranging from (61-77%) in hospitalized and (32-44%) in non-hospitalized patients (22).

CONCLUSION

Results of our study showed high rate of ESBL producing E.coli and Klebsiella isolates both in hospitalized and non-hospitalized patients with UTI. E.coli isolateshad more prevalence than Klebseilla isolates. With regard to low resistance to FM and high resistance to CP, FM can be prescribed in uncomplicated UTI and duo to high rate of resistance to cephalosporins, these antimicrobial agents are not appropriate choices for treatment of complicated UTI. Between parenteral drugs, PTZ, AK and MEM are better choices for this goal. At the end, it seems to make an effective and successful empirical treatment and prevent the emergence

of multidrug resistance organisms, aware of susceptibility patterns obtaining from local data is necessary.

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CONFLICT OF INTERESTS

There is no conflict of interest.

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