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Research Article

Antibiotic resistance pattern of *Klebsiella pneumoniae* isolates producing extended-spectrum beta-lactamases in Zanjan city, Iran

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Abstract

Background and Aim: Extended spectrum beta-lactamases (ESBL) in *Klebsiella pneumoniae* isolates have led to increased antibiotic resistance and mortality in patients. Therefore, the present study was performed to investigate the susceptibility and antibiotic resistance patterns of ESBL- producing *K. pneumoniae* strains isolated from patients referred to Zanjan hospitals.

Materials and Methods: In this descriptive-analytical study of the study of 289 cases of urinary tract infection in Zanjan medical centers in 2019, 100 isolates of *K. pneumoniae* were identified by standard bacteriological methods. Antibiotic susceptibility of the isolates was determined by disk diffusion method and ESBL-producing isolates were identified by combined disk method.

Results: The most resistant to ampicillin (73%) and tetracycline (49%) were the most sensitive to amikacin (90%) and nitrofurantoin (89%), respectively. A total of 40 samples were identified as the final ESBL producer.

Conclusion: Given the high percentage of resistance to third generation cephalosporins, careful antibiograms and avoidance of overuse of antibiotics in infections caused by ESBL-producing organisms is an inevitable necessity.

Keywords: Extended-Spectrum Beta-Lactamases, *K. pneumoniae,* Urinary Tract Infection, Antibiotic Resistance

Introduction

Since sulfanamides and penicillins have come into the field, a new opportunity has emerged in the treatment of diseases. In the early days of the use of these drugs, numerous epidemics subsided. However, infections caused by infectious organisms remain a serious problem (1). There are two important mechanisms through which increased resistance to antibiotics and other drugs. The former is due to spontaneous mutation, in the sense that the mutation occurs at a frequency of about 10 to 5%, altering the susceptibility to the drug, and the drug acts only as a selective agent and promotes the survival of resistant organisms

among organisms (2). The second mechanism of genetic exchange resistance is the genetic information that controls the drug resistance of the bacterium to both chromosomal DNA and extra-chromosomal DNA, ie plasmids, through the transformation, conjugation, and transduction of a (resistant) cell. Transferred to another (sensitive) cell. Hospitalized patients are exposed to nosocomial infections, especially with multidrug-resistant organisms, and are one of the most important contributors to nosocomial infections and as a result mortality from Gram-negative bacilli infection. Since antibiotics, especially in ICU wards, are usually empirically due to the rush of treatment (3-4) ESBLs, with the power to hydrolyze the wide range of beta-lactam antibiotics used in clinics, pose a serious problem in medicine. Bacteria producing ESBLs with class C cephalosporinases encoded by the Amp C chromosomal gene have been the most common mechanism of resistance to Gram-negative bacilli against this antibiotic (5-7). Since the second half of the 1980s, with the reporting of variants of ESBLs and the wide geographical distribution of these enzymes, their release has been discussed as an epidemiological phenomenon (8-9). Urinary tract infections are one of the most common human-acquired infections. In the United States, urinary tract infections are the second most common cause of upper respiratory tract infections, and many men and women are infected throughout their lives. Different factors such as age, sex and immune system influence the prevalence of UTI (10-13). *K. pneumoniae* is one of the gram- negative bacilli of the Enterobacteriaceae family that is distributed in nature and is one of the normal flora bacteria in humans (14- 16). This opportunistic pathogen is responsible for a wide range of infections, especially in hospitalized patients, including septicemia, pneumonia, and urinary tract infections. Colonization of this bacterium is more frequent in hospitalized patients than in outpatients (17-18). Common antibiotics to treat klebsiella infection are mainly beta- lactam drugs. But over-use of these drugs has led to antibiotic resistance to this group of antibiotics in *K. pneumoniae* (19-20). The aim of this study was to evaluate clinical isolates of *K. pneumoniae* collected from hospitals in Zanjan in order to present a sensitivity pattern to experimental antibiotics and phenotypic study of ESBLs producing isolates.

Materials and methods

In this descriptive study, 289 urine samples were collected from outpatients and inpatients of Zanjan hospitals during three months from November to December of 2019 and were cultured on EMB (Merck Company, Germany). Then routine biochemical tests were performed on the colonies. Also, standard strain of *K. pneumoniae* ATCC700603 was used as quality control. Combined disk test was used to evaluate ESBL producing strains. This experiment was performed using ceftazidime (30µg), cefotaxime (30µg), ceftazidime / clavulanic acid (30µg / 10µg) and Cefotaxime / clavulanic acid (30µg / 10µg). For this test, the isolates under study were suspended in physiological saline and their turbidity was adjusted to 0.5 McFarland standard. Then, cotton swabs were cultured in Muller Hinton Agar medium in three directions and after 24 h incubation at 37 ° C, the growth zone diameter was recorded around the discs. Then, cotton swabs were cultured in Muller Hinton Agar medium in three directions and after 24 h incubation at 37 ° C, the growth zone diameter was recorded around the discs. Increase in diameter of more than 5 mm in diameter growth zone around ceftazidime / clavulanic acid (30µg / 10µg) and cefotaxime / clavulanic acid (30µg / 10µg) discs compared to ceftazidime (30µg) and cefotaxime (30µg) discs) Indicates ESBL positive of sample and recorded as positive result. In this experiment *E. coli* ATCC 25922 was used as negative control and *E. coli* ATCC 35218 as positive control. After confirmation of the presence of *K. pneumoniae*, the antibiogram for the samples was recommended by the Clinical and Laboratory Standards Institute. Antibiotic discs used were tetracycline (30 µg), nitrofurantoin (300 µg), ceftazidime (30 µg), ampicillin sulbactam (10 µg), amoxicillin

(25 µg), amoxicillin-clavulanic (25 µg), nalidixic acid (30 µg), amikacin (30 µg), tobramycin (10 µg), imipenem (10 µg), ciprofloxacin (5 µg) and gentamicin (10 µg), (Media Companies). After 24-hour incubation at 37 ° C using a ruler, the growth zone around the discs was measured and compared to the CLSI standards. According to the manufacturer's instructions, the results were based on sensitivity (S) and resistance (R) was reported and semi-susceptible halos were recorded as (I).

Results

In this study, 289 urine samples were collected from 100 (34.60%) *K. pneumoniae*. 60 specimens were isolated from the inpatients ward and 40 samples from the outpatients ward. Based on the results of the combined disk test, 40 samples were identified as final ESBL producers. The results of the sensitivity test against the 12 selected antibiotics are shown in Table1.

Table (1). Frequency of antibiotic resistance pattern of *K. pneumoniae* strains isolated from urinary tract infections

Antibiotics	Resistance	Intermediate	sensitive
Tetracycline	49	10	41
Nitrofurantoin	7	4	89
Ceftazidime	29	29	42
Ampicillin Sulbactam	73	10	17
Amoxicillin	43	16	41
ımoxicillin-Clavulanic	45	0	55
Nalidixic Acid	30	18	52
Amikacin	8	0	92
Tobramycin	18	2	80
Imipenem	21	4	75
Ciprofloxacin	31	3	66
Gentamicin	10	5	85

Discussion

Broad-spectrum beta-lactamases are a group of beta-lactamase enzymes that are of particular importance in antimicrobial therapy. The rate of ESBL production among Enterobacteriaceae varies worldwide (21). In the present study, from 100 *K. pneumoniae* isolates, 60 samples from the inpatient ward and 40 samples from the outpatients ward were isolated. Based on the results of the combined disk test, 40 samples were identified as final ESBL producers. The highest resistance to ampicillin (73%) and tetracycline (49%) were the most sensitive to amikacin (90%) and nitrofurantoin (89%), respectively. However, Shah Cheraghi et al., reported the prevalence of *K. pneumoniae* isolated from urine specimens more than other pathogenic agents (22). In a 2007 study, Amirmozafari and colleagues found that 61.2% of isolates of *K. pneumoniae* had drug resistance. Of these, 20.4% had 100% drug resistance to all cephalosporins (cefexime, ceftriaxone, ceftazidime, etc.) (23). In a paper published by Taslima et al. In 2007 in Bangladesh, resistance to ceftazidime (36%), gentamicin (27%), tetracycline (27%), ciprofloxacin (45%) was reported (24). Feiz Sarshar and Akya showed the highest and lowest resistance to ampicillin and carbapenem antibiotics, respectively, from the 60 isolates tested in 2016. 45% of the isolates were ESBL-producing enzyme. Most of the ESBL enzymes were in hospital isolates (88%) compared to outpatient samples (11%). The highest and lowest resistance were observed to ampicillin and carbapenem antibiotics, respectively (25). Mobasher Kare Jeddi and colleagues showed that 23 (91.43%) isolates were resistant to ceftazidime and 42 (89.26%) isolates were resistant to cefotaxime. Of 47 isolates (97.87%) 46, *K. pneumoniae* isolates were ESBL positive. 100% of *K. pneumoniae* isolates were susceptible to imipenem (26). Sarvazad and Darbouy showed that 60.82 % of cefotaxime resistant isolates, 40.2% ceftriaxone resistant isolates, 62.88% ceftazidime resistant isolates, 3.09% isolates resistant to imipenem, 39.17% of isolates were resistant to cefepime, 64.94% isolates were resistant to cefixime, 26.8% were resistant to amikacin (27). Regional differences in different parts of the world give rise to different antibiotic responses, and even patterns of antibiotic resistance may vary from one hospital to another in one country. The origin of these differences are: genetic differences between individuals, genetic differences of strains, differences in cultural and economic backgrounds. Therefore the treatment pattern used in different regions is different depending on the specific characteristics of a region.

Conclusion

Due to the increased antibiotic resistance among the strains, it is recommended that antibiogram testing be performed before treatment. Also, preventing bacterial strains and therapeutic failures that lead to complication of the infection can be prevented by proper use of existing medicines, completing the course of treatment and avoiding as many antibiotics as possible. Further research in this field will increase our knowledge and more effective exposure to the antibiotic resistance of emerging microorganisms.

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