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Original Research Article

Prevalence of Extended-Spectrum Beta-Lactamases Production in *Escherichia coli* Isolated from Urinary Tract Infection Samples in Zanjan Hospitals, Iran

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Abstract: ***Background and Aim:*** The spread of ESBL in *Escherichia coli* isolates has led to increased antibiotic resistance and mortality. Therefore, the present study was performed to investigate the susceptibility and antibiotic resistance patterns of ESBL-producing *E. coli* strains isolated from patients referred to Zanjan hospitals. ***Materials and Methods:*** In this descriptive-analytical study, 260 urinary tract infection specimens were identified in Zanjan hospitals in 2019, 100 *E. coli* isolates 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 (72%) and tetracycline (50%) were the most sensitive to amikacin (94%) and nitrofurantoin (90%), respectively. A total of 41 samples were identified as the final ESBL producer. ***Conclusion:*** The results indicate a relatively high prevalence of ESBL in *E. coli* isolates in Zanjan. Given the high prevalence rate, it is necessary to monitor the resistance pattern of gram negative isolates in Zanjan. Given the high percentage of resistance to *E. coli* isolates to antibiotics and to reduce the spread of resistance genes, sensitivity testing is recommended to select more effective antibiotics even for outpatient isolates.

Keywords: Extended-Spectrum Beta-Lactamases, *Escherichia coli*, 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 AmpC chromosomal gene have been the most common mechanism of resistance to Gram-negative bacilli against this antibiotic [5, 6]. 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 [7, 8]. 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 [9-12]. *E. coli* is one of the most

common bacterial agents isolated from human infections. The drug resistance of this bacterium is of great importance especially in hospitalized patients [13, 14]. It is one of the most common microbial causes of urinary tract infections and is the cause of many nosocomial infections such as sepsis, wound infections, gastroenteritis and neonatal meningitis [15]. *E. coli* is one of the hospital opportunistic pathogens and has become resistant to beta-lactam antibiotics due to the acquisition of plasmids encoding extended-spectrum beta-lactamases [16, 17]. For this reason, treatment of infections caused by *E. coli* has become difficult [18, 19]. The aim of this study was to evaluate clinical isolates of *E. coli* 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, 260 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 *E. coli* 35218 ATCC 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 Escherichia coli, 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, 260 urine samples were collected from 100 (38.46%) *E. coli*. 64 specimens were isolated from the inpatients ward and 36 samples from the outpatients ward. Based on the results of the combined disk test, 41 samples were identified as final ESBL producers. The results of the sensitivity test against the 12 selected antibiotics are shown in Figure-1.

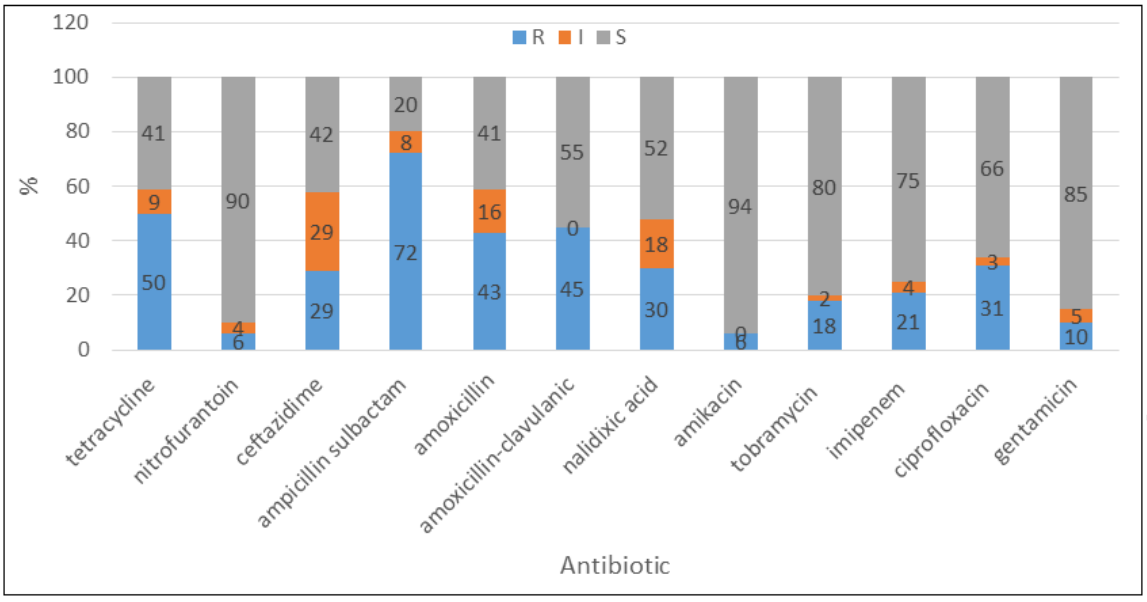

Fig-1: Frequency of antibiotic resistance pattern of *E. coli* strains isolated from urinary tract infections

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 [20]. In the present study, from 100 *E. coli* isolates, 64 samples from the inpatient ward and 36 samples from the outpatients ward were isolated. Based on the results of the combined disk test, 41 samples were identified as final ESBL producers. The highest resistance to ampicillin (72%) and tetracycline (50%) were the most sensitive to amikacin (94%) and nitrofurantoin (90%), respectively. Kasmaei *et al.*, by studying *E. coli* isolated from urinary tract infection, found that the samples were most resistant to ceftriaxone and nalidixic acid (100 %) [21]. Mobasherizadeh *et al.*, found in a study of *E. coli* strains isolated from urinary tract infections that most strains were resistant to ampicillin. Which was consistent with the findings of the present study [22]. 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 [23]. Masjedian and colleagues examined 51% of the 148 *E. coli* strains reported producing ESBL [24]. Mirsalehian and colleagues reported 59.3% of the samples as ESBL producers [25]. In a study by Soltan Dallal *et al.*, on 200 *E. coli* isolates, 64% of the isolates reported ESBL-producing [26, 27]. Amirmozafari and colleagues showed that 2018 out of 167 *E. coli* isolates, 38.9% were ESBL positive [28]. 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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