Evaluation of Antibiotic Resistance Patterns of Clinical *Klebsiella pneumoniae* Isolates from Educational Hospitals in Zahedan, Iran

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Received : 01 Nov 2017
Revised: 07 Dec 2017
Accepted: 16 Dec 2017

ABSTRACT

Background and Objectives: The resistance of gram-negative bacteria to antibiotics has become a serious problem, which imposes a significant increase in treatment costs. *Klebsiella pneumoniae* is an important nosocomial pathogen from the *Enterobacteriaceae* family. The aim of this study was to investigate the frequency and pattern of antibiotic resistance in *K. pneumoniae* strains isolated from clinical samples.

Methods: This descriptive, cross-sectional study was performed on 150 *K. pneumonia* strains isolated from different clinical samples such as urine, sputum, blood, ulcers, lung secretions and abdominal abscess. Antibiogram test was performed using the disk diffusion method (Kirby-Bauer). Minimum inhibitory concentration of amikacin, tobramycin and gentamicin was determined via the E-test for 50 strains with high resistance rates.

Results: In this study, the highest rate of resistance was observed against carbenicilin, ceftriaxone, cefepime and streptomycin. *K. pneumonia* isolates were most frequent in urine and sputum samples. In the E-test, the highest rate of resistance was observed against gentamicin, tobramycin (16µg/ml) and amikacin (64µg/ml).

Conclusion: Based on our results, tigecycline, netilmicin, kanamycin and amikacin are the most effective antibiotics for the treatment of *K. pneumoniae* infections.

Keywords: *Klebsiella pneumoniae*, antimicrobial resistance, E-test method.

This paper should be cited as: Bokaeian M, Shahraki Zahedani SH, Delarampoor A, Atashgah M, Dahmarde B [Evaluation of Antibiotic Resistance Patterns of Clinical *Klebsiella pneumoniae* Isolates from Educational Hospitals in Zahedan, Iran]. mljgoums. 2018; 12(3):41-45

INTRODUCTION

Klebsiella pneumoniae is an important nosocomial pathogen from the *Enterobacteriaceae* family (1). Recent outbreak of infections caused by opportunistic pathogens has become a major challenge in hospitals (2). Similar to some other gram-negative bacteria, *K. pneumoniae* is part of the normal flora of intestine and mouth and can be found as a saprophyte in the respiratory tract of healthy people and even infants (3). Age, chronic bronchopulmonary disease, diabetes and alcoholism are known the risk factors for *K. pneumoniae* infections. Hospital-acquired pneumonia is a severe illness accompanied by rapid bacterial invasion, high fever, bloating, visible abscess on a chest radiograph and mortality rate of 25-50% (4). *K. pneumoniae* is also one of the main causes of wound infections, bacteremia, meningitis and gram-negative UTIs (5, 6). According to Sanches et al., rate of *K. pneumoniae* carriage in hospitalized patients is 77% in the stool, 19% in the pharynx, and 42% on the hands of patients. These high rates were directly related to the use of antibiotics (7). Antimicrobial resistance has always been a major health concern, particularly in hospitals. Alteration of the normal microbial flora by antibiotics provides suitable conditions for the invasion of opportunistic bacteria (8). The high infectivity of *K. pneumoniae* especially in immunocompromised individuals has resulted in some long-term postoperative complications and the excessive use of antibiotics. Therefore, the increasing rate of antibiotic resistance in these bacteria has become a serious health problem (9). Moreover, transfer of antibiotic resistance genes between species has increased the number of antibiotic-resistant species (10). The aim of this study was to study the frequency and antibiotic resistance pattern of *K. pneumoniae* isolates from nosocomial infections samples using the Kirby–Bauer test.

MATERIAL AND METHODS

This descriptive, cross-sectional study was performed on all patients admitted to teaching hospitals of Zahedan (Iran) between July 2016 and October 2016. A total of 250 clinical specimens from urine, sputum, lung secretion, abdominal abscess, blood and ulcers were collected from the patients. The samples were cultured on blood agar, MacConkey agar and eosin-methylene blue agar. The plates were incubated at 37 °C for 24 hours and differential biochemical tests such as TSI, SIM, urease, Simmons' citrate, MR/VP and indole were performed on suspected colonies according to the standard methods. The standard strain of *K. pneumoniae ATCC 700603* was used as positive control. Next, one or two *K. pneumonia*-positive colonies were transferred to vials containing tryptic soy broth containing 10-15% glycerol, and the vials were kept at -20 °C until analysis (11).

Antibiotic resistance pattern of isolates was determined using the Kirby-Bauer disk diffusion method based on the guidelines from the Clinical and Laboratory Standards Institute (CSLI) (12). All antibiotic disks used in the study including amikacin (30μg), gentamicin (10μg), tobramycin (10μg), kanamycin (30μg), netilmicin (30μg), tigecycline (15μg), ceftriaxone (30μg), cefepime (30μg), ciprofloxacin (5μg), carbenicillin (100μg) and streptomycin (10μg) were purchased from the MAST Company, England. Antibiotic susceptibility testing was performed for the isolates and the standard strain of *K. pneumoniae*. Minimum inhibitory concentration (MIC) of gentamicin, amikacin and tobramycin was determined using E-test according to the CLSI guidelines (13). The E-test strips were purchased from the MAST Company, England. Finally, data were analyzed with SPSS (version 18) using chi-square test.

RESULTS

Overall, 150 *K. pneumonia*e isolates were collected in the study. Table 1 shows the frequency of *K. pneumonia* isolates from different clinical specimens. Most *K. pneumonia*e isolates were related to urine (40%) and sputum (30%) samples (Table 1). In addition, the frequency of *K. pneumonia*e was higher in the samples collected from male patients (Figure 1).

The highest rate of resistance was observed against carbenicillin (87.3%), ceftriaxone (31.3%) and cefepime (26.7%). The lowest rate of resistance was observed against kanamycin (6.7%), netilmicin (6%) and *tigecycline* (4%) (Table 2).

Table 1- Frequency of *K. pneumoniae* isolates in different clinical samples

Clinical sample	Number	Percentage
Urine	60	40
Sputum	45	30
Lung secretion	20	13.3
Blood	15	10
Wound	5	3.3
Abdominal abscess	5	3.3
Total	150	100

Figure 1- The frequency distribution of *K. pneumoniae* based on gender

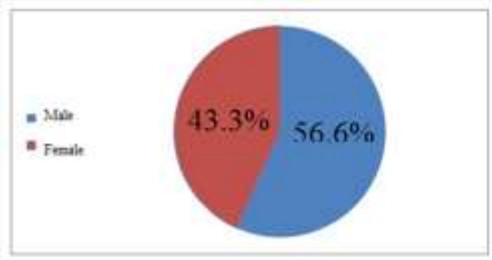

Table 2- The antibiotic resistance patterns of *K. pneumoniae* isolates against various antibiotics

Antibiotic	Number of sensitive isolates (%)	Number of intermediate isolates (%)	Number of resistant isolates (%)
Carbenicillin	8 (5.3%)	11(7.3%)	131(87.3%)
Ceftriaxone	93(64%)	10(6.7%)	47(31.3%)
Cefepime	103(68.7%)	7(4.7%)	40(26.7%)
Streptomycin	96(64%)	17(11.3%)	37(24.7%)
Tobramycin	126(84%)	3(2%)	21(14%)
Ciprofloxacin	101(67.3%)	29(19.3%)	20(13.3%)
Gentamicin	132(88%)	1(0.7%)	17(11.3%)
Amikacin	135(90%)	3(2%)	12(8%)
Kanamycin	120(80%)	20(13.3%)	10(6.7%)
Netilmicin	130(86.7%)	11(7.3%)	9(6%)
Tigecycline	126(84%)	18(12%)	6(4%)

Figure 2- Determination of MIC by the E-test

According to results of the E-test, the minimum and maximum diameters of inhibition zone were 6 and 96 µg/ml for tobramycin, 8 and 96 µg/ml for gentamycin, and 12 and 96 µg/ml for amikacin (Figure 2).

DISCUSSION

Infectious disease and its treatment have been known as serious health challenges, and increased rate of antibiotic resistance has led to a significant rise in the treatment costs (13). *K. pneumoniae*, an important member of the *Enterobacteriaceae* family, is one of the most important causes of nosocomial infections in immunocompromised individuals (14). These infections are often difficult to treat since most causative strains are resistant not only to beta-lactam drugs, but also to aminoglycosides and fluoroquinolones (15, 16). The excessive use of antibiotics has further increased the incidence of antibiotic resistance in bacteria, resulting in treatment failure, development of complications, and increased cost of treatment (17). Because of the genetic variations in the bacterial strains and the difference in the frequency of antibiotic use, a drug resistance rate varies widely in different regions of the world. Hence, identifying important and common hospital pathogens and determining the exact pattern of antibiotic resistance could be of use for the control of outbreaks and reduction of treatment costs (18, 19).

The resistance rates reported in studies from different provinces of Iran have been inconsistent. In study of Behzadian Nejad et al. in Tehran, sensitivity of *K. pneumoniae* isolates to amikacin and gentamicin was found to be 20% and 12.5%, respectively, which are higher than the rates observed in our study (20). In another study in Tehran, the resistance rate against ciprofloxacin, gentamicin and cefepime was 37%, 33% and 40%, respectively (21). In Tehran, Derakhshan et al. found the rate of gentamicin and amikacin resistance to be 60% and 37%, respectively, which are significantly higher than the rates found in the present study (22). However, higher resistance rates to gentamicin and amikacin were reported in two other studies (23, 24). A study in Greece also reported high rate of amikacin resistance (48.2%) in *K. pneumonia* isolates (25). In Pakistan, Ullah et al. reported the resistance rates of *K. pneumoniae* isolates against ciprofloxacin (52.17%), amikacin (32.16%) and gentamicin (80.43%), which are higher than our findings (26). In our study, the frequency of ceftriaxone resistance was 31%, which is higher than the rates reported in studies in the Netherlands (18.7%) and in the USA (10%) (27, 28). The inconsistency in the antibiotic resistance rates might be due to differences in geographical area, type of antibiotics used, type of samples and indiscriminate use of antibiotics (29, 30).

CONCLUSION

Considering the relatively high rate of antibiotic resistance among *K. pneumoniae* strains isolated from the hospitals in the study area, initial identification of these resistant isolates and implementation of appropriate outbreak prevention strategies seem essential. In addition, performing antibiotic sensitivity tests before prescription of drugs would be beneficial for choosing suitable treatment strategies and control of hospital infections and drug resistance.

ACKNOWLEDGMENTS

The present article is part of a master's thesis supported by the Zahedan University of Medical Sciences, Iran. The authors would like to thank the director and personnel of the Research Department for their cooperation.

CONFLICT OF INTEREST

None declared.

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