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Antibiotic resistance pattern of *Klebsiella pneumoniae* isolated from nosocomial infections in Aleshtar hospital, Lorestan province

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Abstract

Introduction: *Klebsiella pneumoniae* is one of *Klebsiella* species. *K. pneumoniae* is one of the most important bacteria causing nosocomial infections. This bacterium threatens public health and leads to increased hospital costs and mortality rate. The purpose of this study was to determine the pattern of antibiotic resistance in *K. pneumoniae* in nosocomial infections.

Methods: This study was performed on 51 samples of *Klebsiella* isolates from 500 patients in three units of Aleshtar hospital in 9 months. The antibiotic resistance of *K. pneumoniae* to 18 antibiotics was performed by Kirby Bauer disk diffusion method.

Results: The frequency of *K. pneumonia* among 500 samples was determined 51 cases (10.2%). The largest number of *K. pneumoniae* was isolated from the infectious unit (49.02%). The frequency of *K. pneumoniae* based on the source of infection for urine was 22 cases (43.14%), sputum 17 (33.33%), stool 6 (11.77%), wound 4 (7.84%), blood 2 (3.92%), and cerebrospinal fluid 0 (0%), respectively. *K. pneumoniae* resistance to antibiotics included: ceftriaxone (94.12%), ciprofloxacin (90.20%), ofloxacin (86.27%), cefotaxime (78.43%), nalidixic acid (58.82%), nitrofurantoin (56.86%), aztreonam (54.90%), ampicillin/sulbactam (50.98%), ticarcillin (45.09%), cefepime (43.14%), colistin (43.14%), gentamicin (41.18%), azithromycin (41.18%), polymyxin B (39.22%), piperacillin/tazobactam (19.61%), amikacin (15.69%), imipenem (5.88%), and meropenem (3.92%).

Conclusion: Meropenem and imipenem with the lowest resistance were the most effective antibiotics against *K .pneumoniae*. Ceftriaxone, and ciprofloxacin antibiotic had the lowest effect on the treatment of *K. pneumoniae*.

Keywords: *Klebsiella pneumoniae*, Antibiotic resistance, Nosocomial infections

Introduction

Nosocomial infections are induced in hospitalized patients. These patients do not have these infections at the time of reference, but are infected at the time of hospitalization (1). Nosocomial infections are caused two days after hospitalization or discharge from the hospital (2). Despite the efforts and measures to control nosocomial infections, economic costs and mortality, are high (3). Nosocomial infections are the sixth cause of death in the United States with 9900 fatalities per annum approximately (4). *Klebsiella pneumoniae* is the most important cause of nosocomial infections among gram-negative bacteria (5). *K. pneumoniae* is an opportunistic pathogen in hospitals, which causes the most common nosocomial infections, such as urinary tract, pneumonia, septicemia, wound, and bloodstream (5). *K. pneumoniae* is gram-negative, rod-shaped, aerobic and facultative anaerobic, non-motile, nitrate positive, with mucoid colonies, and large polysaccharide capsule (6). Four species of the genus *Klebsiella* include *K. pneumoniae*, *K. rhinoscleromatis*, *K. ozaenae*, and *K. oxytoca* that were introduced as pathogens to human (7,8). *K. granulomatis* (called *Calymmatobacterium granulomatis*) also is a human pathogen that is almost unknown (8). *Klebsiella* species, according to the biochemical reactions, can be distinguished from one another (7). One of the major challenges about *K. pneumoniae* is emerging strains resistant to antibiotics (9). Overuse of antibiotics has caused a lot of resistance in the bacteria (9). Approximately 21 000 resistance genes have been identified in bacteria (10). Today, the relationships between the

uses of antibiotics for treatment and antibiotic resistance in *K. pneumoniae* is very clear (10). In 1983, a wide range of extended-spectrum beta-lactamases (ESBLs) for resistance to cephalosporins were reported in *Klebsiella* strains (11). After that, the use of third generation of cephalosporins, such as ceftriaxone, cefotaxime and ceftazidime has been limited to the treatment of infections caused by *K. pneumoniae* (11). In recent years, multi-drug resistance (MDR) in *K. pneumoniae* has been observed around the world (12). *K. pneumoniae* carbapenemases (KPC) is resistant to carbapenem, amino glycosides, cephalosporins, and fluoroquinolones, which usually is sensitive to the colistin antibiotic (12). Indiscriminate use of colistin has led to several resistance cases in *K. pneumoniae* (12). KPC is the most prominent *Enterobacteriaceae* for the production of the enzyme (12). The purpose of this study was to determine the pattern of antibiotic resistance in *K. pneumoniae* by the Kirby Bauer Disk Diffusion (KBDD) method. In this study, the most effective antibiotic to treat *K. pneumoniae* infections has been identified.

Methods

This study was performed on 500 clinical specimens isolated from hospitalized patients in Aleshtar (Lorestan province) in 2014. Urine, stool, blood, sputum, wound, and cerebrospinal fluid (CSF) samples were collected from critical care unit (CCU), infectious, and surgery units at the hospital. Samples were cultured on blood agar (Merck), and Macconkey agar (Merck) media and were incubated at 37°C for 24 hours. Suspected colonies to *Klebsiella* were maintained by the scrutiny of culture media. *Klebsiella* samples were isolated by urease, Simmons citrate, arginine decarboxylase, triple sugar-iron (TSI), and sulfur-indole-motility (SIM) biochemical tests. To identify the *Klebsiella* species were used from Methyl Red/Voges Proskauer (MR/VP), urease, malonate, and SIM differential biochemical tests. Four pathogen *Klebsiella* species were identified from each other by these tests. Each sample was cultured in a different media and *K. pneumoviae* species were isolated after incubation. In order to determine the pattern of antibiotic resistance of *K. pneumoniae*, disk diffusion method was performed. In this case, after the preparation of bacterial suspension turbidity of 0.5 McFarland standard, each sample was cultured on Mueller Hinton agar (Himedia) medium. Then 18 antibiotic disks were placed on the Plates. These included: piperacillin/tazobactam (100/10 µg), ampicillin/sulbactam (10/10 µg), ticarcillin (75 µg), ceftriaxone (30 µg), cefotaxime (75 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), cefepime (30 µg), colistin (10 µg), amikacin (30 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), nitrofurantoin (300 µg), polymyxin B (300 units), azithromycin (15 µg), nalidixic acid (30 µg) and aztreonam (30 µg). Plates were incubated for 24 hours at 37°C. All antibiotic disks were prepared from MAST group. Finally, halos diameter created around each disk were measured based on Clinical Laboratory standards Institute (CLSI 2014) by a ruler and were recorded as resistant (R) intermediate (I), and susceptible (S). SPSS version 20 software was used for data analysis.

Results

Of 500 samples collected from nosocomial infections, 52 samples (10.4%) were for the genus *Klebsiella*, which 51 cases (10.2%) were related to *K. pneumoniae* species. From the 51 samples of *K. pneumoniae*, 35 (68.63%) were for females and 16 (31.37%) for males. P-Value for gender was significant (0.02) and it showed that *Klebsiella* infections were related to the gender. The highest number of frequency for *K. pneumoniae* at the hospital units was related to infectious, surgery, and CCU (Table 1). According to the source of infection, most of *K. pneumoniae* was isolated from urine, sputum, stool, and blood samples, respectively (Table 2). Of the total samples, 52 cases contained *Klebsiella*. Of this number, 51 samples were for *K. pneumoniae*, and 1 case was related to *K. oxytoca* (Table 3). The highest rate of antibiotic resistance with 48 cases (94.12%) was related to ceftriaxone and the lowest was related to 2 cases (3.92%) of meropenem. The number of *K. pneumoniae* of resistant, intermediate, and susceptible are specified to 18 antibiotics tested in Table 4.

Table 1. The frequency of *K. pneumoniae* in hospital units

Hospital Unit	Number	%
Infectious	25	49.02
Surgery	18	35.29
CCU	8	15.69
Total	51	100

CCU, critical care unit

Table 2. The number of *K. pneumoniae* in sites of infection

Site of Infection	Number	%
Urine	22	43.14
Sputum	17	33.33
Stool	6	11.77
Wound	4	7.84
Blood	2	3.92
CSF	0	0.00
Total	51	100

CSF, cerebrospinal fluid

Table 3. The frequency of *K. pneumoniae* among *Klebsiella* species

Klebsiella Spp.	Number	%
K. pneumoniae	51	98.08
K. oxytoca	1	1.92
K. rhinoscleromatis	0	0.00
K. ozaenae	0	0.00
Total	52	100

Table 4. The number of *K. pneumoniae* of resistant, intermediate, and susceptible to 18 antibiotics

Name Disk	No. of Resistant (%)	No. of Intermediate (%)	No. of Susceptible (%)
Ceftriaxone	48 (94.12)	2 (3.92)	1 (1.96)
Ciprofloxacin	46 (90.20)	2 (3.92)	3 (5.88)
Ofloxacin	44 (86.27)	1 (1.96)	6 (11.77)
Cefotaxime	40 (78.43)	3 (5.88)	8 (15.69)
Nalidixic Acid	30 (58.82)	8 (15.69)	13 (25.49)
Nitrofurantoin	29 (56.86)	1 (1.96)	21 (41.18)
Aztreonam	28 (54.90)	4 (7.84)	19 (37.26)
Ampicillin/sulbactam	26 (50.98)	7 (13.73)	18 (35.29)
Ticarcilin	23 (45.09)	6 (11.77)	22 (43.14)
Cefepime	22 (43.14)	9 (17.65)	20 (39.21)
Colistin	22 (43.14)	-	29 (56.86)
Gentamicin	21 (41.18)	10 (19.61)	20 (39.21)
Azithromycin	21 (41.18)	3 (5.88)	27 (52.94)
Polymyxin B	20 (39.21)	-	31 (60.79)
Piperacillin/tazobactam	10 (19.61)	4 (7.84)	37 (72.55)
Amikacin	8 (15.69)	5 (9.80)	38 (74.51)
Imipenem	3 (5.88)	4 (7.84)	44 (86.27)
Meropenem	2 (3.92)	1 (1.96)	48 (94.12)

Discussion

K. pneumoniae is one of the most common bacteria that causes infections in patients (13). Choosing the best antibiotic is very important for the treatment of nosocomial infections caused by *K. pneumoniae*. The use of ineffective antibiotics is the cause of the spread of antibiotic resistance in *Klebsiella* species. In this study, of 500 isolates from nosocomial infections, 51 cases (10.2%) were related to *K. pneumoniae.* This shows the high prevalence of this pathogen in infections. Based on the results obtained in this study, *K. pneumoniae* antibiotic resistance is high. The use of ceftriaxone, ciprofloxacin, and ofloxacin antibiotics for the treatment of infections caused by *K. pneumoniae* not only helps the treatment, but also it causes antibiotic resistance. This study revealed that meropenem, and imipenem are the most effective antibiotics for *K. pneumoniae*. Of 52 isolates of *Klebsiella,* just one sample was related to *K. oxytoca*. This indicates that *K. pneumoniae* is the most common *Klebsiella* species in nosocomial infections. Studies worldwide have determined the frequency of *K. pneumoniae* in nosocomial infection. In a study by Bina et al (14) in 2015, in order to determine *K. pneumoniae* carbapenemase in clinical samples, it was shown that the most antibiotic resistance was for piperacillin (60.6%) and the least resistance was for imipenem (13.9%). In this study, more than 14% of *K. pneumoniae* strains were resistant to carbapenem (14).

Barakzahi et al (15) have specified the antibiotic resistance of *K. pneumoniae* at the hospital of Zahedan in 2014. In this study, the highest numbers of *K. pneumoniae* were isolated from urine and the lowest number from CSF. They also reported that most of antibiotic resistance of *K. pneumoniae* was related to cefixime (82%), and cefotaxime (81%) (15). A study by Feizabadi et al (16) was performed on the antibiotic resistance of *K. pneumoniae* in two hospitals in Tehran in 2007. The highest antibiotic resistance of *K. pneumoniae* was reported for amoxicillin-clavulanic acid (81.81%) and aztreonam (78.78%), and the least resistance was for piperacillin/tazobactam (15.15%) (16). Manjula et al (17) reported that the prevalence of *K. pneumoniae* in urinary tract infections in women was about 10% in southwest India. In this study, the antibiotic resistance of *K. pneumoniae* was determined by Kirby-Bauer disk diffusion. They reported the most *K. pneumoniae* resistance against ampicillin, nitrofurantoin, and cefuroxime.

In a study by Kumar (18) has been determined the antibiotic susceptibility of *K. pneumoniae* isolated from the pus. In this study, of 198 samples, the prevalence of *K. pneumoniae* was 21.1%, and the lowest antibiotic resistance was related to amikacin (11.9%) (18). Kalaskar and Venkataramana (19) have reported *K. pneumoniae* resistance to cephalosporins 1-4 generations close to 100% in 2012. In this study, most of *K. pneumoniae* was isolated from urine (19). Romanus and Egwu (20) examined the antibiotic susceptibility of *K. pneumoniae* in Nigeria in 2011. In this study, in contrast to our study, *K. pneumoniae* sensitivity to all antibiotics was almost 100% (20). Table 5 shows the comparison of *K. pneumoniae* resistance of the present study with other studies.

Conclusion

Antibiotic resistance is worrying in bacteria, and *K. pneumoniae* through the world has become a threat to public health. According to studies conducted in recent years,

Table 5. Comparison of antibiotic resistance of *K. pneumoniae* with other studies

Name Disk	Present Research %	Bina et al (14) %	Barakzahi et al (15) %	Manjula et al (17) %	Kumar 2013 (18) %	Romanus et al 2011 (20) %
Ceftriaxone	94	50	73	-	-	-
Ciprofloxacin	90	-	53	36	88	4
Ofloxacin	86	-	-	-	88	-
Cefotaxime	78	50	67	63	88	3
Nalidixic Acid	58	-	59	-	71	-
Nitrofurantoin	56	-	-	73	-	-
Aztreonam	54	48	-	65	-	2
Ampicillin/Sulbactam	50	-	-	-	57	-
Ticarcilin	45	-	-	-	-	-
Cefepime	43	36	-	53	66	-
Colistin	43	-	-	-	-	-
Gentamicin	41	41	58	36	42	24
Azithromycin	41	-	60	36	-	-
Polymyxin B	39	-	-	21	100	-
Piperacillin/Tazobactam	19	-	-	39	-	-
Amikacin	15	-	64	14	11	0
Imipenem	5	13	43	0	-	0
Meropenem	3	14	-	-	-	-

K. pneumoniae resistance is spread to most antibiotics. In these conditions, *Klebsiella* antibiotic resistance should be determined by tests. This attempt can help to treat *Klebsiella* infections and reduce mortality rate. Although, resistance to multiple antibiotics especially in cases of nosocomial organisms is not new, but it has increased in recent years. The frequency of resistance to other classes of antibiotics such as quinolones and aminoglycosides are also high. Therefore, in the treatment of patients with these multidrug resistance organisms other options including combination therapy or potentially toxic drugs such as polymixin may be used.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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