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iberoamericanjm@gmail.com

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Ayatollahi, Jamshid; Sharifyazdi, Mohammad;  
Fadakarfar, Razieh; Shahcheraghi, Seyed Hossein  
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## Original article

Antibiotic resistance pattern of *Klebsiella pneumoniae* in obtained samples from Ziaee Hospital of Ardakan, Yazd, Iran during 2016 to 2017Jamshid Ayatollahi<sup>a</sup>, Mohammad Sharifyazdi<sup>b</sup>, Razieh Fadakarfar<sup>b,\*</sup>, Seyed Hossein Shahcheraghi<sup>a,\*</sup><sup>a</sup>Infectious Diseases Research Center, Shahid Sadoughi Hospital, Shahid Sadoughi University of Medical Sciences, Yazd, Iran<sup>b</sup>Medical Student, Shahid Sadoughi Hospital, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

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## ABSTRACT

**Background:** In recent years, due to the inappropriate use of antibiotics, drug resistance has increased in gram negative bacilli, including *Klebsiella pneumoniae*. Drug resistance is associated with an increase in mortality and therapeutic costs. Therefore, determination of an antibiotic resistance pattern for choosing the appropriate treatment for infections caused by this bacterium seems necessary. This study was conducted to determine the antibiotic resistance pattern of *Klebsiella pneumoniae* species isolated from patients referring to Ziaee Hospital in Ardakan in 2016-2017.

**Materials and Methods:** For this descriptive-analytic study, all positive cultures of *Klebsiella pneumoniae* in patients referred to Ziaee Hospital in Ardakan during 2016 to 2017, were evaluated. Antibiotic resistance patterns of the samples were determined by the standard method of propagation of the disk from 12 different antibiotics and data analyzed by SPSS 21 software.

**Results and discussions:** The results of this study, which were performed on 75 samples, showed that 22 (29.3%) were male and 53 (70.7%) were female. *Klebsiella's* resistance to clarithromycin was 100%, but was 100% susceptible to amikacin. The percentage of *Klebsiella's* resistance to ampicillin was 78.3%, cefalotin 75%, cotrimoxazole 43.9%, ceftriaxone 32%, ciprofloxacin 30.9%, cefotaxime 24%, and ampicillin 20%. The highest sensitivity of *Klebsiella pneumoniae* for antibiotics was 100% for amikacin, 82.8% for meropenem and 82% for cefepime. Due to the high prevalence of resistance in *Klebsiella* samples, there is a need for strict measures in the administration of antibiotics. Antibiotic resistance can also be reduced by choosing the appropriate antibiotic for treatment and by taking antibiotic susceptibility tests.

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## 1. BACKGROUND

In recent years, antibiotics have been used to treat disease-causing bacteria. Antimicrobial resistance is a global threat

to public health [1-3].

Enzymatic modification (b-lactamases), reduced permeability (selective blockage), increased membrane transport (efflux pumps), altered binding site (specific receptors) and metabolic bypass are the mechanisms of

\* Corresponding author.

E-mail address: [shahcheraghi@gmail.com](mailto:shahcheraghi@gmail.com) and [fadarazie@gmail.com](mailto:fadarazie@gmail.com)

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antibiotic resistance in bacteria [4-6].

The detection of multidrug resistance is, however, a primary step in effectively controlling antibiotic-resistant bacterial infections that can lead to clinical failure and additional antibiotic resistance [7-9].

The gram-negative organism of *Klebsiella pneumoniae* (*K. pneumoniae*) is one of the most important causes of nosocomial infections in developing countries and one of the eight most important causes in developed countries [10-13]. Nosocomial infections caused by *K. pneumoniae* multi-antibiotic resistant have become an increasing public health concern. *K. pneumoniae* produces various pathogenic virulence factors, including capsular polysaccharide, lipopolysaccharide, fimbriae, siderophores and resistance [14-16].

Increased global infections by multidrug-resistant bacteria closely associated with limited drug treatments imply the failure of empirical treatments and strengthen the need for antibacterial therapies based on antimicrobial sensitivity tests [1, 17, 18].

Several studies have demonstrated outbreaks in hospitals due to *K. pneumoniae* resistant to cephalosporins, aminoglycosides and quinolones of third generation [10]. Therefore, this study aimed to investigate the antimicrobial susceptibility profile of *Klebsiella pneumoniae* isolates collected during the period of January 2016 till December 2017 at Ziaee Hospital in Ardakan.

## 2. MATERIALS AND METHODS

From January 2016 to December 2017 all positive cultures of *Klebsiella pneumoniae* in patients referred to Ziaee Hospital in Ardakan during 2016 and 2017, were evaluated. This study was approved by ethical committee of Yazd University of medical sciences with ID: IR.SSU.MEDICINE.REC.1395.278. It was result of thesis of general physician.

In this cross-sectional study, the samples were collected as census from all positive cultures of *K. pneumoniae* by a checklist that was previously provided by the researcher and confirmed by qualified specialists.

Samples were obtained in a sequential census of all positive cultures in terms of *K. pneumoniae* at the designated time period.

Data collection was carried out based on a questionnaire that was previously designed in terms of gender, age, the type of antibiotic (including: imipenem, meropenem,

cefepim, ciprofloxacin, amikacin, ceftazidime, ceftriaxon, cefotaxime, ampicillin, cotrimoxazol, cefalotin and clarithromycin) and finally the type of sample (including: urine, blood, ulcer, sputum, pleural fluid, urethral discharge, eye secretions and fecal). Inclusion criteria were including all positive cultures for *Klebsiella pneumoniae*. The cases in which sample data was incomplete were excluded from study and were as exclusion criteria.

In this study, the disc diffusion sensitivity test was used to evaluate the sensitivity and resistance of *K. pneumoniae* to antibiotics. In the disc diffusion method, a certain amount of bacteria is set according to the existing standards in terms of the degree of dilution and has already been identified. Special culture media add to the same plates in terms of diameter, depth, etc., and discs the standardized antibiotic filter paper is placed on the plate surface. After the time it takes to grow the microbes, if the antibiotic is able to prevent the growth of the microorganism, it does not grow around the bacterial disk, and the bacteria to the antibody the biotype is more sensitive, these will be no larger growth halo.

Detection and differentiation of sensitive, semi-sensitive and resistant conditions from each other was performed based on the diameter of the around the colony as millimeters and according to the relevant table in laboratory. The data were collected, recorded in SPSS software (version 21) and analyzed by chi-Square test.

## 3. RESULTS AND DISCUSSIONS

Of 75 samples, 22 (29.3%) were related to men and 53 (70.7%) were for women. Of these, 60 patients (80%) were admitted and 15 patients (20%) were outpatient.

The samples were including urine specimens that were highest numbers with 64 (85.3%) and then, sputum 4 (5.3%), wound and blood each one 3 (4%) and finally secretions 1 (1.3%).

*Klebsiella* resistance was 100% for clarithromycin but about amikacin was susceptible in 100% of cases (Table 1). It should be noted that for all specimens, all antibiotic disks have not been used. For each sample, according to the type of specimen and physician's opinion, a certain number of disks were inserted. For example, clarithromycin was used in only 6 cases.

**Table 1. The frequency distribution of *Klebsiella pneumoniae* susceptibility based on the type of antibiotic**

| Antibiotic     | Resistant |      | Semi-sensitive |     | Sensitive |      | Total     |
|----------------|-----------|------|----------------|-----|-----------|------|-----------|
|                | N         | %    | N              | %   | N         | %    |           |
| Amikacin       | 0         | 0    | 0              | 0   | 18        | 100  | 18 (100%) |
| Meropenem      | 4         | 13.8 | 1              | 3.4 | 24        | 82.8 | 29 (100%) |
| Cefepim        | 8         | 16   | 1              | 2   | 41        | 82   | 50 (100%) |
| Ceftazidime    | 4         | 12.5 | 2              | 6.3 | 26        | 81.3 | 32 (100%) |
| Imipenem       | 7         | 20   | 1              | 2.9 | 27        | 77.1 | 35 (100%) |
| Cefotaxime     | 6         | 24   | 0              | 0   | 19        | 76   | 25 (100%) |
| Ciprofloxacin  | 21        | 30.9 | 1              | 1.5 | 46        | 67.6 | 68 (100%) |
| Ceftriaxon     | 16        | 32   | 2              | 4   | 32        | 64   | 50 (100%) |
| Cotrimoxazol   | 18        | 43.9 | 0              | 0   | 23        | 56.1 | 41 (100%) |
| Cefalotin      | 6         | 75   | 0              | 0   | 2         | 25   | 8 (100%)  |
| Ampicilin      | 18        | 78.3 | 1              | 4.3 | 4         | 17.4 | 23 (100%) |
| Clarithromycin | 6         | 100  | 0              | 0   | 0         | 0    | 0 (100%)  |

N: Number of sample; \* Significance susceptibility based on gender.

In women, the highest sensitivity was observed for from the Hospital Universitário de Santa Maria, Brazil for

**Table 2. The frequency distribution of *Klebsiella pneumoniae* susceptibility based on gender**

| Gender         | Woman     |    |                |   |           |    | Man       |    |                |   |           |    | * p-value |
|----------------|-----------|----|----------------|---|-----------|----|-----------|----|----------------|---|-----------|----|-----------|
| Result         | Resistant |    | Semi-sensitive |   | Sensitive |    | Resistant |    | Semi-sensitive |   | Sensitive |    |           |
| Antibiotic     | %         | N  | %              | N | %         | N  | %         | N  | %              | N | %         | N  |           |
| Amikacin       | 0         | 0  | 0              | 0 | 100       | 11 | 0         | 0  | 0              | 0 | 100       | 7  | 0.51      |
| Meropenem      | 11.8      | 2  | 0              | 0 | 88.2      | 15 | 16.7      | 2  | 8/3            | 1 | 75        | 9  | 0.43      |
| Cefepim        | 9.4       | 3  | 3.1            | 1 | 87.5      | 28 | 27.8      | 5  | 0              | 0 | 72.2      | 13 | 0.18      |
| Ceftazidime    | 8.7       | 2  | 4.3            | 1 | 87        | 20 | 22.2      | 2  | 11.1           | 1 | 66.7      | 6  | 0.41      |
| Imipenem       | 13        | 3  | 4.3            | 1 | 82.6      | 19 | 33.3      | 4  | 0              | 0 | 66.7      | 8  | 0.30      |
| Cefotaxime     | 23.1      | 3  | 0              | 0 | 76.9      | 10 | 25        | 3  | 0              | 0 | 75        | 9  | 0.91      |
| Ciprofloxacin  | 20.8      | 10 | 2.1            | 1 | 77.1      | 37 | 55        | 11 | 0              | 0 | 45        | 9  | 0.01      |
| Ceftriaxon     | 25.7      | 9  | 2.9            | 1 | 71.4      | 25 | 46.7      | 7  | 6.7            | 1 | 46.7      | 7  | 0.24      |
| Cotrimoxazol   | 40        | 12 | 0              | 0 | 60        | 18 | 54.5      | 6  | 0              | 0 | 45.5      | 5  | 0.40      |
| Cefalotin      | 71.4      | 5  | 0              | 0 | 28.6      | 2  | 100       | 1  | 0              | 0 | 0         | 0  | 0.53      |
| Ampicilin      | 75        | 15 | 5              | 1 | 20        | 4  | 100       | 3  | 0              | 0 | 0         | 0  | 0.61      |
| Clarithromycin | 100       | 5  | 0              | 0 | 0         | 0  | 100       | 1  | 0              | 0 | 0         | 0  | 0.55      |

N: Number of sample; \* Significance susceptibility based on gender.

amikacin (100%) and meropenem (88.2%). In the case of ciprofloxacin, there was a significant difference between the sexes ( $p=0.01$ ). In men, resistance to cefalotin, ampicillin and clarithromycin antibiotics was 100%, but in women only resistance to clarithromycin was 100%. Overall, the resistance to all antibiotics examined in men was higher than women (Table 2).

In total, the resistance to all antibiotics examined was in the hospitalized patients more than outpatient cases, but this difference was significant only for meropenem ( $p=0.001$ ) and was probably due to a small number of outpatient cases (Table 3).

Body secretions (Other than ulcers, blood, sputum, urine) were resistant to chloramphenicol and ampicillin (100%), and 100% sensitive to cotrimoxazole, cefotaxime and ceftriaxone. The frequency distribution of *Klebsiella pneumoniae* sensitivity based on of sample type has been listed in Table 4.

A study assessed the 1805 *Klebsiella pneumoniae* isolates

antimicrobial susceptibility profile. Resistance to colistin (239.3%), ciprofloxacin (64%), and amikacin (21.4%) was found increased [1]. In the present study, the sensitivity rate was the highest against amikacin. Also the sensitivity rate to ciprofloxacin (67.6%) was more than mentioned study.

Another study showed that among the 168 *K. pneumoniae* isolates studied, 72.0% were resistant to ceftazidime, almost 69% to cefotaxime and 67.2% to amikacin [10]. In our study the sensitivity was 100% against amikacin and there was no any resistance to it.

In a study that was performed on *Klebsiella pneumoniae* isolates from the hospitals in sari, Mazandaran, the highest resistance against cefotaxime (100%) and ceftazidime (100%) was observed among all isolates, while the highest susceptibility to gentamycin was observed (63%) [19]. But at present study, the highest sensitivity and resistance of *Klebsiella pneumoniae* for antibiotics was related to amikacin and clarithromycin, respectively.

**Table 3. The frequency distribution of *Klebsiella pneumoniae* susceptibility based on type of admission**

| Type of admission | Outpatient |   |                    |   |           |   | Hospitalized |    |                    |   |           |    | * p-value |
|-------------------|------------|---|--------------------|---|-----------|---|--------------|----|--------------------|---|-----------|----|-----------|
| Result            | Resistant  |   | Semi-sensitiv<br>e |   | Sensitive |   | Resistant    |    | Semi-sensitiv<br>e |   | Sensitive |    |           |
| Antibiotic        | %          | N | %                  | N | %         | N | %            | N  | %                  | N | %         | N  |           |
| Amikacin          | 0          | 0 | 0                  | 0 | 100       | 4 | 0            | 0  | 0                  | 0 | 100       | 14 | 0.45      |
| Meropenem         | 0          | 0 | 100                | 1 | 0         | 0 | 14.3         | 4  | 0                  | 0 | 85.7      | 24 | 0.001     |
| Cefepim           | 16.7       | 1 | 0                  | 0 | 83.3      | 5 | 15.9         | 7  | 2.3                | 1 | 81.8      | 36 | 0.93      |
| Ceftazidime       | 0          | 0 | 20                 | 1 | 80        | 4 | 14.8         | 4  | 3.7                | 1 | 81.5      | 22 | 0.28      |
| Imipenem          | 25         | 1 | 0                  | 0 | 75        | 3 | 19.4         | 6  | 3.2                | 1 | 77.4      | 24 | 0.91      |
| Cefotaxime        | 0          | 0 | 0                  | 0 | 100       | 4 | 28.6         | 6  | 0                  | 0 | 71.4      | 15 | 0.22      |
| Ciprofloxacin     | 30.8       | 4 | 0                  | 0 | 69.2      | 9 | 30.9         | 17 | 1.8                | 1 | 67.3      | 37 | 0.88      |
| Ceftriaxon        | 37.5       | 3 | 0                  | 0 | 62.5      | 5 | 31           | 13 | 4.8                | 2 | 64.3      | 27 | 0.78      |
| Cotrimoxazol      | 41.7       | 5 | 0                  | 0 | 58.3      | 7 | 44.8         | 13 | 0                  | 0 | 55.2      | 16 | 0.85      |
| Cefalotin         | 0          | 0 | 0                  | 0 | 0         | 0 | 75           | 6  | 0                  | 0 | 25        | 2  | 0.51      |
| Ampicilin         | 60         | 3 | 0                  | 0 | 40        | 2 | 83.3         | 15 | 5.6                | 1 | 11.1      | 2  | 0.29      |
| Clarithromycin    | 0          | 0 | 0                  | 0 | 0         | 0 | 100          | 4  | 0                  | 0 | 0         | 0  | 0.34      |

N: Number of sample; \* Significance susceptibility based on gender.

An investigation was conducted to investigate the prevalence of *K. pneumoniae* isolates and their antibiotic susceptibility pattern. The highest number of *K. pneumoniae* were resistant to ampicillin (75.6%) followed

by, nitrofurantoin and cefuroxime almost 74% and least to chloramphenicol (13%) [20]. In the present study, the highest sensitivity was related to amikacin.

**Table 4. The frequency distribution of *Klebsiella pneumoniae* susceptibility based on type of admission**

| Type of sample | Sputum    |           |          | Wound    |           |          | Blood    |           |          | Urine     |           |           | * p-value |
|----------------|-----------|-----------|----------|----------|-----------|----------|----------|-----------|----------|-----------|-----------|-----------|-----------|
| Result         | Sens.     | Semi-sens | Resist.  | Sens.    | Semi-sens | Resist.  | Sens.    | Semi-sens | Resist.  | Sens.     | Semi-sens | Resist.   |           |
| Antibiotic     | N (%)     | N (%)     | N (%)    | N (%)    | N (%)     | N (%)    | N (%)    | N (%)     | N (%)    | N (%)     | N (%)     | N (%)     |           |
| Amikacin       | 2 (100)   | 0 (0)     | 0 (0)    | 1 (100)  | 0 (0)     | 0 (0)    | 1 (100)  | 0 (0)     | 0 (0)    | 14 (100)  | 0 (0)     | 0 (0)     | 0.41      |
| Meropenem      | 1 (50)    | 0 (0)     | 1 (50)   | 0 (0)    | 0 (0)     | 1 (100)  | 1 (50)   | 0 (0)     | 1 (50)   | 22 (91.7) | 3 (4.2)   | 1 (3.3)   | 0.05      |
| Cefepim        | 3 (100)   | 0 (0)     | 0 (0)    | 1 (100)  | 0 (0)     | 0 (0)    | 2 (66.7) | 0 (0)     | 1 (33.3) | 35 (81.4) | 1 (2.3)   | 7 (26.3)  | 0.95      |
| Ceftazidime    | 15 (83.3) | 1 (5.6)   | 2 (11.1) | 1 (100)  | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     | 1 (100)  | 24 (85.7) | 1 (3.6)   | 3 (10.7)  | 0.02      |
| Imipenem       | 3 (75)    | 0 (0)     | 1 (25)   | 1 (50)   | 0 (0)     | 1 (50)   | 1 (50)   | 0 (0)     | 1 (50)   | 22 (81.5) | 1 (3.7)   | 4 (14.8)  | 0.81      |
| Cefotaxime     | 1 (100)   | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     | 0 (0)    | 1 (100)  | 0 (0)     | 0 (0)    | 17 (77.3) | 0 (0)     | 5 (22.7)  | 0.28      |
| Ciprofloxacin  | 1 (50)    | 0 (0)     | 1 (50)   | 1 (33.3) | 0 (0)     | 2 (66.7) | 2 (66.7) | 0 (0)     | 1 (33.3) | 42 (70)   | 1 (1.7)   | 17 (28.3) | 0.87      |
| Ceftriaxon     | 1 (33.3)  | 0 (0)     | 2 (66.7) | 1 (50)   | 0 (0)     | 1 (50)   | 1 (33.3) | 1 (33.3)  | 1 (33.3) | 27 (65.9) | 1 (2.4)   | 13 (31.7) | 0.44      |
| Cotrimoxazol   | 1 (50)    | 0 (0)     | 1 (50)   | 0 (0)    | 0 (0)     | 1 (100)  | 1 (100)  | 0 (0)     | 0 (0)    | 20 (57.1) | 0 (0)     | 15 (42.9) | 0.38      |
| Cefalotin      | 0 (0)     | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     | 1 (100)  | 2 (28.6)  | 0 (0)     | 5 (71.4)  | 0.53      |
| Ampicilin      | 1 (100)   | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     | 1 (100)  | 0 (0)    | 0 (0)     | 1 (100)  | 4 (21.1)  | 1 (5.3)   | 14 (73.6) | 0.99      |
| Clarithromycin | 0 (0)     | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     | 1 (100)  | 0 (0)    | 0 (0)     | 2 (100)  | 0 (0)     | 0 (0)     | 2 (100)   | 0.55      |

N: Number of sample; \* Significance susceptibility based on gender.

## 4. SUMMARY

The results of our study showed that the highest sensitivity of *Klebsiella pneumoniae* for antibiotics was to amikacin (100%). Also, *Klebsiella*'s resistance to clarithromycin was 100%. The resistance to all antibiotics examined was in the hospitalized patients more than outpatient cases, but this difference was significant only for meropenem. Also, the resistance to all antibiotics examined in men was higher than women.

## 5. CONCLUSION

Currently, *K. pneumoniae* has reached high levels of endemicity in hospitals and is one of the main causes of nosocomial outbreaks. The results on our study show that *Klebsiella*'s resistance to clarithromycin was 100%, but was 100% susceptible to amikacin. It needs to investigate in the more cities in our province for preventing resistant infections by organism.

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