Open Access Publish Free

http shgri.com

Report of Health Care

Original Article

Volume 1, Issue 1, 2015, p. 1-5

The frequency and pattern of antibiotic resistance among *Klebsiella* spp. isolated from nosocomial infection in Khorramabad hospital

Sajad Babakhani[1], Shahram Shokri[2*], Mohammadreza Nazer[3]

[1]MSc of Microbiology, Department of Microbiology, Islamic Azad University, Ashkezar Branch, Yazd, Iran
[2]Assistant Professor, Infectious Disease Specialists, Lorestan University of Medical Sciences, Lorestan, Iran
[3]Associate Professor, Infectious Disease Specialists, Lorestan University of Medical Sciences, Lorestan, Iran

Received: 7 June 2014
Accepted: 28 August 2014
Published online: 19 December 2014

***Corresponding author:** Shahram Shokri, School of Medicine, Lorestan University of Medical Sciences, Lorestan, Iran. Email: shahram35040@yahoo.com

Competing interests: The authors declare that no competing interests exist.

Citation: Babakhani S, Shokri S, Nazer M. The frequency and pattern of antibiotic resistance among *Klebsiella* spp. isolated from nosocomial infection in Khorramabad hospital. Report of Health Care 2015; 1(1): 1-5.

Abstract

Introduction: Nosocomial infections are primarily caused by *Klebsiella* bacteria, which lead to an increase in healthcare costs and the mortality rate. Concerning the alarming increased antibiotic resistance of *Klebsiella* species, the purpose of this study was to compare the frequency and determine the antibiotic resistance pattern of *Klebsiella* species isolated from nosocomial infections using Kirby Bauer method.

Methods: This study was done on 80 specimens of *Klebsiella* isolates from 480 hospitalized patients in three different units of Khorramabad Shohadaye Ashayer hospital. After determining the bacterial species, antibiotic-resistance test of *Klebsiella* species was performed for 15 antibiotics using the disk diffusion method. The results were recorded based on Clinical and Laboratory Standards Institute (CLSI) standard guidelines.

Results: The frequency rates of *Klebsiella* species were determined as follows: *Klebsiella pneumoniae* (91%), *Klebsiella oxytoca* (5%), *Klebsiella rhinoscleromatis* (3%), and *Klebsiella ozaenae* (1%). The frequency of *Klebsiella* species on the basis of sources of infection and type of sample respectively were: urine (42%), lung and sputum (36%), wound (15%), blood (6%), and cerebro-spinal fluid (CSF) (1%). The resistance of all *Klebsiella* species to studied antibiotics were respectively as follows: ceftriaxone (92%), ciprofloxacin (82%), nitrofurantoin (80%), ofloxacin (75%), cefotaxime (70%), imipenem (67%), ticarcillin (66%), nalidixic acid (60%), gentamicin (52%), azithromycin (40%), cefepime (31%), polymyxin B (22%), colistin (17%), amikacin (7%), and meropenem (1%).

Conclusion: According to the obtained results, meropenem and amikacin with the lowest resistance are the most effective antibiotics against all *Klebsiella* strains, and although ceftriaxone antibiotic does not help in the treatment of *Klebsiella* infections, but it may cause antibiotic resistance.

Keywords: *Klebsiella*, Infection, Antibiotic resistance

Introduction

Nosocomial infection is among the most difficult problems confronting physicians and patients (1). This infection is caused two days after hospital admission or within two days after hospital discharge (2). Almost all pathogenic bacteria have the potential to cause infection in hospitalized patients, but only limited number of both gram-positive and gram-negative bacteria cause the majority of nosocomial infections (3).

Gram-negative bacteria bacilli cause the four most frequent types of nosocomial infections, including Pneumonia, Surgical Site Infections (SSI), Urinary Tract Infection (UTI), and Blood Stream Infections (BSI) (4). *Klebsiella* species isolated from different infections, the most common and important species of which is *Klebsiella pneumonia*, are Pathogenic bacteria that cause urinary tract infections, pneumonia, septicemia, wound infections and 7% of nosocomial infections caused by bacteria (5). Some *Klebsiella* bacteria are found naturally in the human digestive tract and in some cases, such as immune deficiency, can cause deadly diseases (6).

Klebsiella bacteria are gram-negative, medium rod-shaped, non motile, facultative aerobic and anaerobic Fermentative, catalase positive, Lactose negative, reducing nitrate to nitrite, mucoid growth and have a large polysaccharide capsule (6,7). Reports suggested that *Klebsiella* is emerging worldwide and has become a serious threat to human health by causing endemic and epidemic infections (8). *Klebsiella pneumoniae*, *Klebsiella granulomatis*, and *Klebsiella oxytoca* are three species of the genus *Klebsiella* in which *K.granulomatis* is almost an unknown bacterium and it is also called *Calymmatobacterium granulomatis*.

The other two species of the genus *Klebsiella* by the names of *Klebsiella rhinoscleromatis* and *Klebsiella ozaenae* cause infection in the lungs and are considered as subspecies of *Klebsiella pneumoniae* (9).

Today, not only great progress has been made in approaches to treat the infections, but also some advancement have been made in the production of powerful antibiotics. Therefore, most bacteria have become resistant against these antibiotics. This has made the treatment of infectious diseases very difficult (10). Now we are facing a challenge due to the development of antibiotic resistance in *Klebsiella* species and the production of multidrug-resistant strains improvement from hospital-acquired infections (11). In 1980, the resistance of *Klebsiella* strains to the cephalosporins such as oxyimino beta-lactams (ceftriaxone, ceftazidime, cefotaxime) was reported and recorded for the first time and since then *Klebsiella* bacteria are more resistant to antibiotics (12).

Klebsiella pneumoniae carbapenemase (KPC) has become resistant against nearly all antibiotics, but it shows sensitivity to colistin antibiotic, however unfortunately, in recent years as a consequence of the increased use of colistin, *K.pneumoniae* has become resistant to this antibiotic. Reports such as colistin-resistant K.pneumoniae ST258 isolated in Italy support this matter (13). *Klebsiella* bacteria were so resistant to antibiotics that the first meropenem-resistant and imipenem-resistant strains of *K.pneumoniae* were reported in 2001 (14). Serotyping of *Klebsiella* species is performed on the basis of the classification of capsular antigens or specifications O, K antigens. Capsule typing method is difficult and time-consuming and requires expensive anti-capsular antiserum that is not much available (15).

The purpose of the present study was to determine the antibiotic resistance pattern of *Klebsiella* spp. isolated from nosocomial infection in Khorramabad Shohadaye Ashayer hospital using Kirby Bauer Disk Diffusion (KBDD) method. This study tends to determine the most effective antibiotic against *Klebsiella* species and help physicians, patients, and hospitals to reduce the mortality rates from nosocomial infections caused by *Klebsiella* species and also to reduce healthcare costs.

Methods

This descriptive study was performed on 480 clinical samples isolated from hospitalized patients in Khorramabad Shohadaye Ashayer hospital during April to September 2013.

The clinical samples (urine, blood, wound, sputum and lung, and Cerebrospinal Fluid (CSF)) were collected from Intensive Care Unit (ICU), Infectious Unit (IFU), surgical unit, and they were cultured on blood agar and MacConkey agar (Merck, Merck) media then incubated at 37 °C for 24 hours. Then, differential biochemical tests such as Urease, Simmons citrate, arginine and ornithine decarboxylase, Sulfur Indole Motility (SIM), and Triple Sugar Iron Agar (TSI) were performed on grown colonies suspicious of *Klebsiella* bacteria and according to standard tables *Klebsiella* bacteria were identified. Following this stage, differential biochemical tests, such as Methyl Red (MR), Voges-Proskauer (VP), urease, and malonate were done to identify *Klebsiella* species. After culturing the *Klebsiella* colonies on differential media, they were incubated for 24 hours at 37 °C and *Klebsiella* species were identified in accordance with standard tables.

The antibiotic resistance of *Klebsiella* was examined using KBDD method. After preparing the bacterial suspension with 0.5 McFarland turbidity standard, some of the samples were removed using sterile swab and were cultured in several directions on Mueller-Hinton agar (Himedia) medium. The following antibiotic disks from MAST group were placed on culture media: cefotaxime (75 µg), ceftriaxone (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), polymyxin B (300 U), colistin (10 µg), gentamicin (10 µg), amikacin (30 µg), imipenem (10 µg), meropenem (10 µg), nitrofurantoin (300 µg), azithromycin (15 µg), nalidixic acid (30 µg), and ticarcilin (75 µg). Then plates were incubated at 37 °C for 24 hours.

To examine the resistance rate, the halos diameters created around each disk were measured by a ruler and were recorded as sensitive (S), intermediate (I), and resistance (R) on the basis of the Clinical and Laboratory Standards Institute (CLSI) 2013. SPSS v.19 software was used to analyze the data.

Results

According to the results obtained from 480 samples examined for nosocomial infections, 80 cases were associated with Klebsiella, which included 16.6% of the total samples. Of 80 positive samples, 40 cases (61.3%) and 31 cases (38.7%) were related to females and males respectively. The highest number of *Klebsiella* was isolated from intensive care unit, infectious unit, and surgery unit consecutively. The frequency percentage of *Klebsiella* in each of these hospital units is presented in Table 1. The clinical specimens from which *Klebsiella* was isolated in order of frequency were urine, lungs and sputum, wound, blood, and CSF (Table 2). *Klebsiella* species in terms of frequency included *pneumoniae, oxytoca, rhinoscleromatis*, and *ozaenae* (Table 3). The highest antibiotic resistance in *Klebsiella* was related, respectively, to ceftriaxone (third generation cephalosporins) with 74 cases (92.5%) and ciprofloxacin (fluoroquinolones) with 66 cases (82.5%). This shows that these antibiotics have no effect on the treatment of infections caused by *Klebsiella.* They even increase the antibiotic resistance to *Klebsiella*. The lowest resistance rate was associated respectively with meropenem antibiotic with 1 case (1.2%) and amikacin with 6 cases (7.5%). This highlights that meropenem is highly effective in treating *Klebsiella* infections. Those *Klebsiella* that were resistant, intermediate, and susceptible to 15 antibiotics were examined and the resistance percentage of each antibiotic is presented in Table 4.

Discussion

In this study of 480 clinical specimens examined for

hospital-acquired infections, 80 samples (16.6%) were associated with *Klebsiella* bacteria. This high frequency of *Klebsiella* in nosocomial infections is noticeable. *Klebsiella* species are typically resistant to most antibiotics. For this reason a correct choice and use of antibiotics is important for the treatment of *Klebsiella* infection and even if recovery is achieved, it is necessary to finish the course of antibiotics use. Conserning the prevalence of arbitrary use of antibiotics, it is important to perform tests for determining antibiotic resistance to pathogenic bacteria. The highest number of *Klebsiella* was isolated from urine and the lowest number was related, respectively, to CSF and blood, demonstrating this bacterium less enters of the blood and CSF.

Table 1. The number and percentage frequency of *Klebsiella* in hospital units

Hospital unit	Number	%
Intensive Care	46	57.5
Infectious	20	25
Surgery	14	17.5

Table 2. The number and percentage frequency of *Klebsiella* in different sites of infection

Site of infection	Number	%
Urine	33	41.3
Lungs and sputum	29	36.3
Wound	12	15
Blood	5	6.2
CSF	1	1.2

CSF= Cerebrospinal fluid

Table 3. The number and percentage frequency of *Klebsiella* species

Klebsiella species	Number	%
K.pneumoniae	73	91.3
K.oxytoca	4	5
K.rhinoscleromatis	2	2.5
K.ozaenae	1	1.2

Based on the results obtained in this study, *Klebsiella* possesses high antibiotic resistance. This resistance to imipenem antibiotic is 67%, which is considered a high resistance for Klebsiella. However, regarding two antibiotics of meropenem and amikacin, the resistance of *Klebsiella* was respectively, 1% and 7%, which indicates that these antibiotics will act effectively against Klebsiella.

In a study conducted by Soltan Dalal *et al* in Imam Khomeini hospital in Tehran, the frequency rate of *Klebsiella* in nosocomial infections was reported 25%. This is an indication of the high presence of this bacterium in hospital-acquired infections. In line with the present study, *K.pneumoniae* had the highest frequency rate (94%) and *K.ozaenae* and *K.rhinoscleromatis* had the lowest frequency rate among *Klebsiella* species. Soltan Dalal *et al* reported the highest resistance of *Klebsiella* against amoxicillin antibiotic (97%) and the lowest resistance against amikacin (0%), While Langarizadeh *et al* in a study on *Klebsiella pneumoniae* in urinary tract infections in Tabriz reported the highest rate of resistance of *Klebsiella pneumoniae* to amoxicillin antibiotic (98.61%) and the lowest resistance to imipenem (20.83%) (16,17). These findings are inconsistent with the findings of our study.

In a study carried out by Mohammadimehr *et al* in Khanevadeh and Golestan hospital in Tehran in 2007, the highest resistance of *Klebsiella* was related to amikacin (96%) and ampicillin (96%), and the lowest resistance to imipenem antibiotic (8%), cefotaxime/clavulanic acid (11%), and nitrofurantoin (13%). These results are different from our findings and other studies (18). The investigation carried out by Al-Shara in Jordan, the highest susceptibility of *Klebsiella* pneumoniae was reported for ciprofloxacin (90.5%) and the lowest susceptibility was considered for ampicillin (16.6%) and amoxicillin-clavulanic acid (22.5%) (19).

In a study by Sarathbabu *et al* in 2012, the highest number of *Klebsiella* was isolated from urine and the lowest resistance was against amikacin antibiotic and highest

Table 4. The percentage of resistance and number *Klebsiella* of resistant, intermediate and susceptible to 15 antibiotics

Name disk	Susceptible	Intermediate	Resistant	Resistance %
Cefotaxime	21	3	56	70
Ceftriaxone	2	4	74	92.5
Cefepime	41	14	25	31.2
Ciprofloxacin	11	3	66	82.5
Ofloxacin	18	2	60	75
Polymyxin B	62	-	18	22.5
Colistin	66	-	14	17.5
Gentamicin	26	12	42	52.5
Amikacin	67	7	6	7.5
Imipenem	22	4	54	67.5
Meropenem	77	2	1	1.2
Nitrofurantoin	14	2	64	80
Azithromycin	42	6	32	40
Nalidixic acid	16	16	48	60
Ticarcilin	18	9	53	66.2

Table 5. The comparison of antibiogram pattern of *Klebsiella* species

Antibiotics	Present reaserh %	Al-Shara *et al* (19) %	Amin et al (20) %	Soltan dalal *et al* (16) %	Langarizadeh *et al.* (17) %	Mohammadimehr et al (18) %	Sedighian et al (21) %	Shajari et al. (22)
Cefotaxime	70	46	82	-	-	88	57	74
Ceftriaxone	92	55	85	-	-	92	62	-
Cefepime	31	-	-	-	-	88	-	-
Ciprofloxacin	82	10	55	-	43	68	63	32
Ofloxacin	75	-	47	-	-	-	-	-
Polymyxin B	22	-	-	-	-	-	-	-
Colistin	17	-	-	55	-	-	-	-
Gentamicin	52	43	-	30	74	48	54	50
Amikacin	7	31	-	0	43	96	44	13
Imipenem	67	30	7	2	21	8	-	40
Meropenem	1	-	7	-	-	-	-	-
Nitrofurantoin	80	-	-	44	94	52	-	-
Azithromycin	40	-	-	-	-	-	-	-
Nalidixic acid	60	34	42	2	58	-	-	75
Ticarcilin	66	-	-	-	-	-	-	-

resistance was against tetracycline (23). In another study, Olajide *et al* investigated the antimicrobial susceptibility pattern of *Klebsiella* species using disk diffusion method in Nigeria. In this study, *Klebsiella* species showed 0% resistance to foxapen, erythromycin, and doxycycline and highest resistance to amoxicillin (75%). The results of this study are in contrast to our findings and other studies (24). Table 5 provides a comparison of the results from the antibiotic pattern of *Klebsiella* species in our study with other studies.

Conclusion

Although resistance to multiple antibiotics, especially in cases of nosocomial organisms is not new but it has increased in recent years. Nowadays, for empirical treatment of nosocomial infections carbapenems (e.g., imipenem, meropenem) and cephalosporines consistently are used but in our study resistance to these antibiotics are remarkable. The frequency of resistance to other classes of antibiotics such as quinolones and aminoglycosides are also high. Therefore, for treatment of patients with these multi-drug resistance organisms other options including combination therapy or potentially toxic drugs such as polymixin may be used. Treatment of patients with such infections is difficult and sometimes impossible. Decrease in this trend of antibiotic resistance maybe reachable through antibiotic stewardship programs. Although, especial local or federal stewardship program has not been settled, yet, we propose to provide and accomplishing such programs in order to decreasing pattern of antibiotic resistance.

Acknowledgments

The authors of this study would like to thank and express sincere appreciation to officials and staff of the laboratory of Khorramabad Shohadaye-Ashayer hospital, especially microbiology unit and all those who helped us to conduct this study.

Ethical issues

Not applicable.

References

1. Jain A, Singh K. Recent advances in the management of nosocomial infection. Journal of Medical Education and Research (JK Sciences) 2007; 9(1): 3-8.
2. Shaikh JM, Devrajani BR, Shah SZ, Akhund T, Bibi I. Frequency, pattern and etiology of nosocomial infection in intensive care unit: an experience at a tertiary care hospital. J Ayub Med Coll Abbottabad 2008; 20(4): 37-40.
3. Bereket W, Hemalatha K, Getenet B, Wondwossen T, Solomon A, Zeynudin A, et al. Update on bacterial nosocomial infections. Eur Rev Med Pharmacol Sci 2012; 16(8): 1039-44.
4. Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis 2005; 41(6): 848-54.
5. Ullah F, Malik SA, Ahmed J. Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiella pneumoniae from urinary tract infections in the North-West of Pakistan. Afr J Microbiol Res 2009; 3(11): 676-80.
6. Siri GP, Sithebe NP, Ateba CN. Identification of Klebsiella species isolated from modimola dam (Mafikeng) north west province-south Africa. Afr J Microbiol Res 2011; 5(23): 3958-63.
7. Brooks GF. Enteric gram-negative rods (Enterobacteriaceae). In: Brooks GF, Butel GS, Morse SA, editors. Jawetz, Melnick, & Adelberg's Medical Microbiology. 23th ed. USA: McGraw-Hill; 2004. p. 248-61.
8. Sikarwar AS, Batra HV. Challenge to health care: multidrug resistance in Klebsiella pneumoniae.

International Proceeding of Chemical, Biological and Enviromental Engineering 2012; 9: 130-4.
9. Donnenberg MS. Enterobacteriaceae. Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 7th ed. Philadelphia, Pennsylvania: Churchill Livingstone Elsevier; 2010. p. 218.
10. Karamoddini MK, Fazli-Bazzaz BS, Emamipour F, Ghannad MS, Jahanshahi AR, Saed N, *et al.* Antimicrobial efficacy of lytic bacteriophages against antibiotic-resistant Klebsiella species. Scientific World Journal 2011; 11: 1332-40.
11. Sikarwar AS, Batra HV. Prevalence of antimicrobial drug resistance of Klebsiella pneumoniae in India. International Journal of Bioscience, Biochemistry and Bioinfoormatics 2010; 1(3): 211-5.
12. Romanus II, Egwu OA, Ngozi AT, Chidiebube NA, Chika EP. Extended spectrum beta-lactamase(ESBL) mediated resistance to antibiotics among Klebsiella pneumoniae in Enugu Metropolis. Maced J Med Sci 2009; 2(3): 196-9.
13. Comandatore F, Sassera D, Ambretti S, Landini MP, Daffonchio D, Marone P, *et al.* Draft genome sequences of two multidrug resistant Klebsiella pneumoniae ST258 isolates resistant to colistin. Genome Announc 2013; 1(1): 1-2.
14. Chen H, Yuehua X, Shen W, Zhou H, Zhou L, Li Z. Epidemiology and resistance mechanisms to imipenem in Klebsiella pneumoniae: a multicenter study. Mol Med Rep 2013; 7(1): 21-5.
15. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and Pathogenicity Factors. Clin Microbiol Rev 1998; 11(4): 589-603.
16. Soltan Dalal MM, Miremadi SA, Sharify Yazdi MK, Rastegar Lari A, Rajabi Z, Yans SA. Antimicrobial resistance trends of Klebsiella spp. isolated from patients in Imam Khomeini hospital. Payavard Salamat Journal 2012; 6(4): 275-81. [In Persian]
17. Langarizadeh N, Ahangarzadeh Rezaee M, Aghazadeh M, Hasani A. Prevalence of multi-drug resistant (MDR) klebsiella pneumoniae among children and adults with urinary tract infection referred to Tabriz teaching hospitals. Quarterly Journal of Biological Sciences 2010; 4(1): 9-17. [In Persian]
18. Mohammadimehr M, Feizabadi MM, Bahadori A. Antibiotic resistance pattern of gram negative bacilli caused nosocomial infections in ICUs in Khanevadeh and Golestan hospital in Tehran -2007. Annals of Military And Health Sciences Research 2011; 8(4): 283-90. [In Persian]
19. Al-Shara M. A 5 year (2005-2009) review of antimicrobial susceptibility of clinical Klebsiella pneumoniae isolates from pediatric patients in Jordan. Al Ameen J Med Sci 2013; 6(4): 316-9.
20. Amin A, Ghumro P, Hussain S, Hameed A. Prevalence of antibiotic resistance among clinical isolates of Klebsiella pneumoniae isolated from a Tertiary Care Hospital in Pakistan. Malaysian Journal of Microbiology 2009; 5(2): 81-6.
21. Saeidynia F, Keihanian F, Saeidynia A. Antibiotic resistance in blood culture samples from patients referred to Razi Laboratory of Rasht, 2006-2011. Advances in Infectious Diseases 2014; 4: 165-72. [In Persian]
22. Shajari G, Khorshid AI, Moosavi G. Bacterial isolation and antibiotic resistance of nosocomial pneumonia in hospitalaized patients - Kashan Iran.Journal of Hormozgan University of Medical Sciences 2009; 13(3): 197-205. [In Persian]
23. Sarathbabu R, Ramani TV, Bhaskara rao K, Panda S. Antibiotic susceptibility pattern of Klebsiella pneumoniae isolated from sputum, urine and pus samples. Journal of Pharmacy and Biological Sciences 2012; 1(2): 04-09.
24. Olajide FA, Ameh JA, Simon MK. Antibiotic resistance and susceptibility pattern/profile of Staphylococcus aureus and Klebsiella species to popularly – used and readily available antibiotics in Zaria, Nigeria. Journal of Phytotherapy and Pharmacology 2012; 1(4): 16-21.