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ORIGINAL ARTICLE

Comparison frequency and Determination antibiotic resistance pattern of *Klebsiella* SPP. isolated from Nosocomial infection in Khorramabad Shohadaye Ashayer hospital

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ABSTRACT
Nosocomial infections are primarily caused by Klebsiella bacteria, which leads to an increase in health care costs and mortality rate. Concerning the alarming increased antibiotic resistance of Klebsiella species, the purpose of study was to compare frequency and determine antibiotic resistance pattern of Klebsiella species isolated from nosocomial infections using Kirby Bauer method. This study was done on 80 specimens of Klebsiella isolates from 480 hospitalized patients in Three different units 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 and results Were recorded based on CLSI standard guidelins. Frequency rates of Klebsiella species were determined as follows: Klebsiella pneumoniae (91%), Klebsiella oxytoca (5%), Klebsiella rhinoscleromatis (3%) And Klebsiella ozaenae (1%). Frequency of Klebsiella species on the basis of source of infection and type of sample respectively were: urine (42 %), lung and sputum (36%), wound (15%), blood (6%) and CSF (1%). Resistance of all Klebsiella species to studied antibiotics are 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%), meropenem (1%). According to the obtained results, meropenem and amikacin with lowest resistance are the most effective antibiotics against all Klebsiella strains, and ceftriaxone antibiotic not only will not help treat Klebsiella infections, however may also causes antibiotic resistance.
Key words: *Klebsiella, Infection, Antibiotic Resistance*

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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 being hospital discharge [2]. Almost all pathogenic bacteria has 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 are cause 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 suggest 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* that *K. granulomatis* is almost an unknown bacterium and other name for it is *Calymmatobacterium granulomatis*. Two other 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, although great progress has been made in approaches to treatment of infections and production of powerful antibiotics, most bacteria have been resistant against these antibiotics, which has made the treatment of infectious diseases very difficult [10]. Due to the development of Antibiotic resistance in *Klebsiella* species and the Production of multidrug-resistant strains improvement from hospital-acquired infections caused by these strains has become a challenge (11). In 1980, the resistant 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 were ever more resistant to antibiotics [12].

Klebsiella pneumoniae carbapenemase (KPC) has become resistant against nearly all antibiotics, but 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* was 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 present study is to determine antibiotic resistance pattern of *Klebsiella* spp. isolated from nosocomial infection in Khorramabad Shohadaye Ashayer hospital using Kirby Bauer disk diffusion method (KBDD). This study tends to determine the most effective antibiotic against *Klebsiella* species and help physicians, patients, and hospital reduce mortality rates from nosocomial infections caused by *Klebsiella* species and also reduces health care costs.

MATERIALS AND METHODS

This descriptive study has been 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 cerebro-spinal 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. After this time, 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) and 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 recording with standard tables.

antibiotic resistance of *Klebsiella* was examined using disk diffusion (Kirby-Bauer) method. After preparing the bacterial suspension with 0.5 McFarland turbidity standard, some of each sample 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 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 clinical and laboratory standards institute (CLSI) 2013. SPSS v.19 software was used to analyze data from.

RESULTS

According to the results obtained from 480 samples examined for nosocomial infections, 80 cases were associated with *Klebsiella*, which includes 16.6% of total samples.

Of 80 positive samples, 40 cases (61.3%) were related to females and 31 cases (38.7%) related to males. The highest number of *Klebsiella* were isolated respectively from intensive care unit, infectious unit,

and surgery unit. The frequency percentage of *Klebsiella* in each of these hospital units is presented in table 1. Clinical specimens from which *Klebsiella* was isolated in order of frequency are urine, lungs and sputum, wound, blood, and CSF (table 2).

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

Hospital unit	Number	Frequency %
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	Frequency %
Urine	33	41.3
Lungs and sputum	29	36.3
Wound	12	15
Blood	5	6.2
CSF	1	1.2

Klebsiella species in terms frequency are include *pneumoniae*, *oxytoca*, *rhinoscleromatis* and *ozaenae* (table 3).

Table 3 : Number and percentage frequency of *Klebsiella* species

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

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%), which shows that these antibiotics have no effect on the treatment of infections caused by *Klebsiella*, even increase 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%) that indicates meropenem is highly effective in treating *Klebsiella* infections. The number of *Klebsiella* that were resistant, intermediate, and susceptible to 15 antibiotics were examined and resistance percentage of each antibiotic is presented in table 4 and diagram 1.

Table 4 : Percentage of resistance and number *Klebsiella* of resistant, intermediate and susceptible to 15 antibiotics

Name disk	Susceptible(N)	Intermediate(N)	Resistant(N)	Resistance%	(p.valeu)
Cefotaxime	21	3	56	70	0.996
Ceftriaxone	2	4	74	92.5	0.996
Cefepime	41	14	25	31.2	0.010
Ciprofloxacin	11	3	66	82.5	0.951
Ofloxacin	18	2	60	75	0.862
Polymyxin B	62	-	18	22.5	0.010
Colistin	66	-	14	17.5	0.005
Gentamicin	26	12	42	52.5	0.326
Amikacin	67	7	6	7.5	0.014

Imipenem	22	4	54	67.5	0.718
Meropenem	77	2	1	1.2	0.005
Nitrofurantoin	14	2	64	80	0.927
Azithromycin	42	6	32	40	0.074
Nalidixic acid	16	16	48	60	0.529
Ticarcilin	18	9	53	66.2	0.689

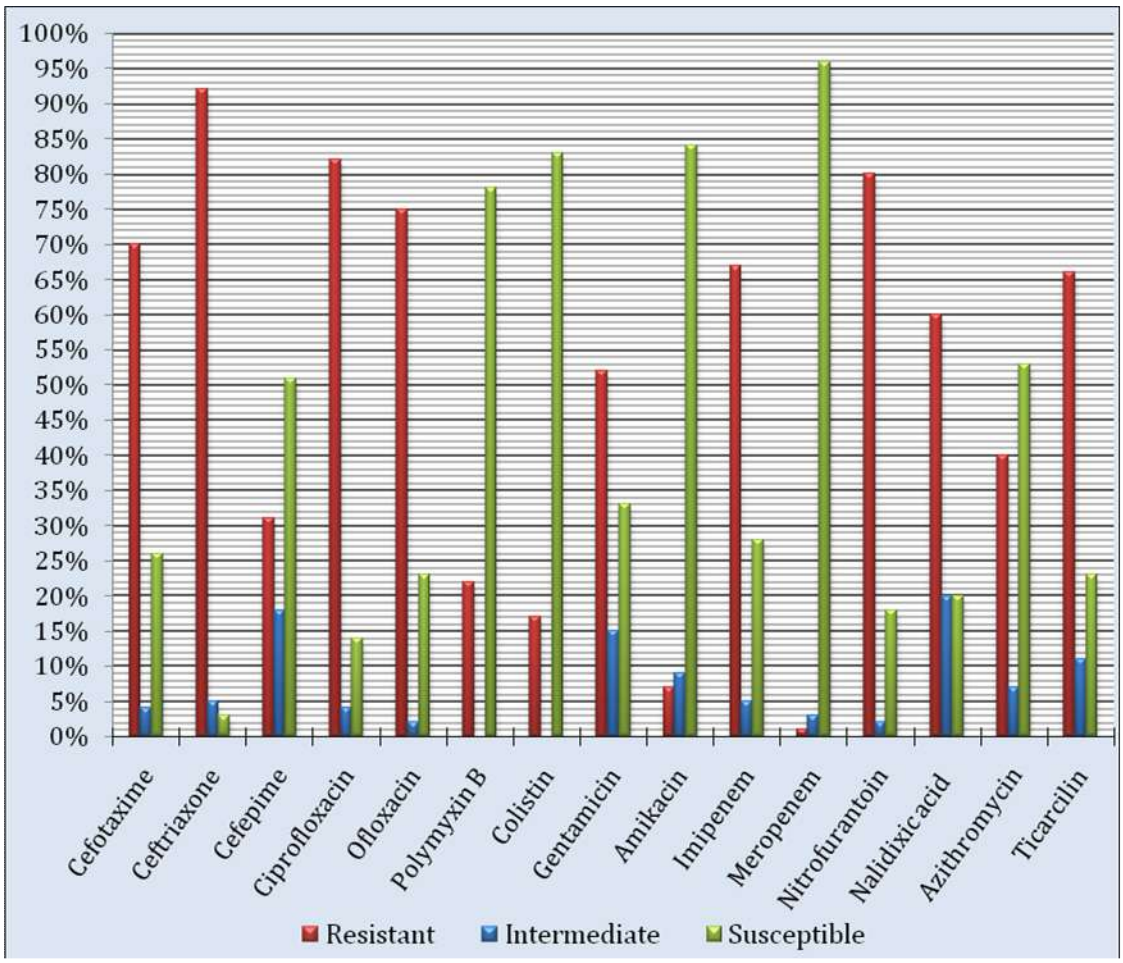

diagram 1 Percentage of resistance *Klebsiella* of resistant, intermediate and susceptible to 15 antibiotics

DISCUSSION AND CONCLUSION

In this study, of 480 clinical specimens examined for hospital-acquired infections, 80 samples (16.6%) were associated with *Klebsiella* bacteria that can be concluded 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 treatment of *Klebsiella* infection is important and even if recovery is achieved, it is necessary to finish the course of antibiotics use. Conserning the prevalence 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 cerebro-spinal fluid (CSF) and blood, demonstrating this bacterium less enters of the blood and CSF.

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

In a study conducted by Soltan Dallal et al. (2012) in Imam Khomeini hospital in Tehran, the frequency rate of *Klebsiella* in nosocomial infections was reported 25% that is an indication of high presence of this bacterium in hospital-acquired infections. In line 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 Dallal et al. reported the highest resistance of *Klebsiella* against amoxicillin antibiotic (97%) and The lowest resistance against amikacin (0%). While Langarizadeh et al.

(2010) in a study on *Klebsiella pneumoniae* in urinary tract infections in Tabriz have reported the highest rate of resistance of *Klebsiella pneumoniae* to amoxicillin antibiotic (98.61%) and The lowest resistance to imipenem (20.83%), which are not consistent with our results [16,17].
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 et al. (2013) in Jordan were reported the highest susceptibility of *Klebsiella pneumoniae* to ciprofloxacin (90.5%) and Lowest susceptibility to ampicillin (16.6%) and *amoxicillin-clavulanic acid* (22.5%) (19).
In a study done by Sarathbabu et al. in 2012, the highest number of *Klebsiella* were isolated from urine and the lowest resistance against amikacin antibiotic and highest resistance against tetracycline were observed [20]. In another study, Olajide et al. [21] investigated 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%) that results of this study are in contrast to our findings and other studies . Table 5 provides a comparison of results from antibiotic pattern of *Klebsiella* species in our study with other studies.

Table 5 : Comparison of antibiogram pattern of *Klebsiella* species other research with our study

Antibiotics	Present reaserh %	Al-shara et al. in 2013 %	Amin et al. in 2009 %	Soltan dallal et al. in 2012 %	Langarizadeh et al. in 2010 %	Mohammadimehr et al. in 2007 %	Shajari et al. in 2006 %
Cefotaxime	70	46	82	-	-	88	74
Ceftriaxone	92	55	85	-	-	92	-
Cefepime	31	-	-	-	-	88	-
Ciprofloxacin	82	10	55	-	43	68	32
Ofloxacin	75	-	47	-	-	-	-
Polymyxin B	22	-	-	-	-	-	-
Colistin	17	-	-	55	-	-	-
Gentamicin	52	43	-	30	74	48	50
Amikacin	7	31	-	0	43	96	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	-	-	-	-	-	-

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REFERENCES
1. Jain, A., Singh. K., (2007). Recent advances in the management of nosocomial infection. *Journal of Medical Education and Research(JK Sciences),* 9(1), 3-8.
2. Shaikh, J. M., Devrajani, B.R., ZulfiqarAlishah, S., Akhund, T., Bibi, I., (2008). Frequency, pattern and etiology of nosocomial infection in intensive care unit: An experience at a tertiary care hospital. *Journal of Ayub Medical College Abbottabad(JAMC),* 20(4), 37-40.
3. Bereket, W., et al., (2012). Update on bacterial nosocomial infection. *European Review for Medical and Pharmacological Sciences,* 16, 1039-1044.

4. Gaynes, R., Edwards, J.R., 2005. Overview of nosocomial infections caused by gram-negative bacilli. *Healthcare Epidemiology,* 41, 848-854.
5. Ullah, F., Malik, S.A., Ahmed, J., (2009). Antimicrobial resistance of *Klebsiella pneumoniae* from urinary tract infection in the north –west of Pakistan. *African Journal of Microbiology Research,* 3(11), 676-680.
6. Siri, G. P., Sithebe, N.P., Ateba, C.N., (2011). Identification of *Klebsiella* species isolated from modimola dam (Mafikeng) north west province-south Africa. *African Journal of Microbiology Research,* 5(23), 3958-3963.
7. 7 .Brooks, G. F., (2004). Enteric gram-negative rods *(Enterobacteriaceae)*. *In:* Brooks, G. F., Butel, G.S., Morse, S.A., ed. *Jawetz, Melnick, & Adelberg's Medical Microbiology.* 23 ed.: Lange Medical Books, 248-261.
8. Sikarwar, A. S., Batra, H.V., (2012). Challenge to health care: multidrug resistance in *Klebsiella pneumoniae*. *International Proceeding of Chemical, Biological and Enviromental Engineering(IPCBEE),* 9, 130-134.
9. Donnenberg, M. S.,(2010). *Enterobacteriaceae*. *In:* Bennett, J. E., et al. ed. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases.* 218.
10. Khajeh Karamoddini, M., et al., (2011). Antimicrobial efficacy of lytic bacteriophages against antibiotic-resistant *Klebsiella* species. *The Scientific World Journal(TSWJ),* 11, 1332-1340.
11. Sikarwar, A. S., Batra, H.V., 2011. Prevalence of antimicrobial drug resistance of *Klebsiella pneumoniae* in India. *International Journal of Bioscience,Biochemistry and Bioinfoormatics(IJBBB),* 1(3), 211-215.
12. Romanus, I. I., Egwu, O.A., Ngozi, A.T., Chidiebube, N.A., Chika, E.P., (2009). Extended spectrum beta-lactamase(ESBL) mediated resistance to antibiotics among *Klebsiella pneumoniae* in Enugu Metropolis. *Macedonian Journal of Medical Sciences(MJMS),* 2(3), 196-199.
13. Comandatore, F., et al., 2013. Draft genome sequences of two multidrug resistant *Klebsiella pneumoniae* ST258 isolates resistant to colistin. *Genome Announcements,* 1(1), 1-2.
14. Chen, H., et al., 2013. Epidemiology and resistance mechanisms to imipenem in *Klebsiella pneumoniae*: A multicenter study. *Molecular Medicine Reports,* 7, 21-25.
15. Podschun, R., Ullmann, U., 1998. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and Pathogenicity Factors. *Clinical Microbiology Reviews,* 11(4), 589–603.
16. Soltan Dalal, M.M., ET AL., (2012). Antimicrobial resistance trends of *Klebsiella* spp. isolated from patients in Imam Khomeini hospital. *Journal of Faculty of Tehran University of Medical Sciences Payavard Salamat*, 6(4), 275-281.
17. Langarizadeh, N., et al., (2010). Prevalence of multi-drug resistant (MDR) *Klebsiella pneumoniae* among children and adults with urinary tract infection referred to tabriz teaching hospitals. *Journal of Biological Sciences, Islamic Azad University, Zanjan*, 4(1), 9-17.
18. Mohammadimehr, M., Feizabadi, MM., Bahadori, A., (2010). Antibiotic resistance pattern of gram negative bacilli caused nosocomial infections in ICUs in Khanevadeh and Golestan hospital in Tehran -2007. *Journal Of Ardabil University Of Medical Sciences*(*JAUMS*), 8(4), 283-290.
19. Al-Shara, M., 2013. A 5 year (2005-2009) review of antimicrobial susceptibility of clinical *Klebsiella pneumoniae* isolates from pediatric patients in Jordan. *Al Ameen Journal of Medical Sciences,* 6(4), 316-319.
20. Sarathbabu, R., et al., (2012. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples. *Journal of Pharmacy and Biological Sciences,* 1(2), 04-09.
21. Olajide, F. A., Ameh, J. A., Simon, M.K., 2012. 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,* 1(4), 16-21.

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