Internal Medicine and Medical Investigation Journal
E-ISSN: 7750-2474
Homepage: www.imminv.com

ORIGINAL ARTICLE

Study of Klebsiella Pneumonia Antibiotic-resistance of K1 and K2 Serotypes in Nosocomial Infections with Hospital Origin

Rezvan Doustipour[1], Fatemeh Keshavarzi[1*], Fariba Lahourpour[2]

[1]*Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*

[2]*Department of Pathology and Medical Laboratory Sciences, Kurdistan University of Medical Science, Sanandaj, Iran*

***Corresponding Author:** Fatemeh Keshavarzi, E-mail: gol.keshavarzi@gmail.com

ARTICLE INFO

Article history
Received: July 12, 2019
Accepted: Oct 12, 2019
Published: Dec 25, 2019
Volume: 4
Issue: 4

Key words:
Klebsiella Pneumonia, Antibiotic Resistance, Hospital-acquired Infection, K2, K1

ABSTRACT

Introduction: *Klebsiella pneumonia* are the most pollutants of the hospital origin. The aim of this study was to determine the antibiotic resistance pattern of Klebsiella pneumonia in two serotypes K1 and K2 from samples collected with urinary tract infections and burn injuries in Kermanshah of Iran. **Materials and Methods:** This study was performed on 140 samples collected from hospitals in Kermanshah for a period of 6 months. After confirmation of bacteria by phenotypic method, genotyping was done by PCR of rmpA2 and magA1 genes. Subsequently, Antibiotic resistance pattern was evaluated according to the CLSI 2017 regimen using 6 types of antibiotic disks. **Results:** The results of genotype determination showed that 76% of the samples were related to K1 serotype and 24% of the samples were related to K2 serotype. In addition, among the samples taken from the 35 urine, K1 showed an 80% prevalence (28 samples) and 20% were related to K2. Of the 35 samples examined from burn injuries, 71% of the samples were related to K1 and 29% related to K2. The antibiogram results showed that the samples of K1 positive were resistant to Onloxacin and Nitrofurantoin antibiotics and sensitive to Ceftoxime and limit to Ceftriaxone antibiotics. The interface has the Trimethoprim and Cefazolin antibiotics. In addition, K2 serotype is susceptible to Ceftoxime and resistant to Nitrofurantoin and intermediate mode for Cefazolin, Forloxacin and Trimethoprim antibiotics. Additionally, the resistance k1 serotype is higher than k2. **Conclusion:** In both samples of urine and burn in the studied population, the prevalence of serotype k1 was higher and in both groups, the most resistant to antibiotics were Nitrofurantoin and onloxacin. In addition, the most commonly used antibiotics that are recommended are Cefotaxime and Ceftriaxone, and resistant antibiotics that should be used less than Nitrofurantoin and Onloxacin.

INTRODUCTION

Klebsiella pneumonia is considered as one of the most important opportunistic pathogens that causes a wide range of diseases with a hospital origin, including pneumonia, urinary tract infections and bacteremia in hospitalized patients (1). This bacterium accounts for between 75% and 86% of the population of Klebsiella, accounting for more than 90% of hospital-acquired infections and is responsible for the prevalence of infections associated with the hospital environment (2). Antibody resistance Microbial strains of Klebsiella are one of the most important concerns in the world One of the results is the high rate of widespread use of antibiotics in medical treatments in developing countries (3). In addition, resistance to broad-spectrum antibiotics (ESBL) has also been frequently reported among strains of the bacterium (4). Reports indicate that Klebsiella pneumonia is one of the most important pathogenic microorganisms, which tend to be widespread. Various pathogenicity causes these bacteria to overwhelm the immune system of mammals against infection, one of which is the polysaccharide capsule present in the bacterial level, which itself has an effect on its pathogenicity it is very important (5). The greatest potential for developing this bacterium is in the respiratory tract, urinary tract, blood and ulcers. Studies show that the K1 and K2 capsules are more prevalent in this bacterium (6). The ability of this organism to increase the incidence of disease due to the reduction in host defense as a result of complex and prolonged surgical procedures or the use of different drugs is increasing (7). In recent years, there have been several reports of the progression of resistance to antibiotics in gram-negative pathogens (8). However, no significant information is available on the source of transmission and on the transmission of bacteria in hospital infections. In

Published by Mehrabani Publishing LLC.

http://dx.doi.org/10.24200/imminv.v3i4.18

addition, epidemiological studies of Klebsiella infections due to different methods and dispersion of these microbes and the lack of generalized methods in all laboratories to determine the types of these bacteria is not a problem. Serology and tests for capillary swelling of different serotypes of Klebsiella are methods used to determine the type of cluster (9). The polysaccharide capsules produced by most species of this bacterium are one of the most important pathogens among the species. To date, 78 serotypes have been identified (serotype K) (10), which vary depending on the severity of the disease. In the meanwhile, isolates of serotype K1 and K2 exhibit the highest incidence and severity of disease compared to other non-capsular isolates (11).

It has also been reported that among all identified serotypes, K1 and K2 serotypes are one of the most important medical conditions for resistance to ampicillin and anti-phagocytosis and serum resistance, while other antibodies identified for serotypes ordinary sensitization (12). Therefore, considering the above mentioned and biological and ecological map of Klebsiella infections and its diffusion, the study of the resistance of this Gram-negative organism isolated from clinical specimens is important in determining the drug resistance in two important serotypes of pathogenicity K1 and K2. (13).

The aim of this study was to determine the antibiotic resistance pattern of Klebsiella species K1 and K2 isolated from samples from hospital infections in Kermanshah.

MATERIALS AND METHODS

This study is a descriptive study. Samples from 70 patients hospitalized in the burns section of Kermanshah hospitals with confirmed Klebsiella infection were sampled. At first, the samples were cultured in Eosin methylene blue (EMB). In order to determine the bacterial species, the samples obtained in EMB medium were sampled in differential culture media of Methyl Red/Voges-Proskauer (MR-VP), Urea agar base, Citrate, Sulfur Indole Motility Media (SIM) and triple sugar iron (TSI). The media used for the assay were purchased from the German MARCK Company. All cultivating conditions. They were purchased from Sina Clone Company of Tehran (SINACLONEBIOCOMPANY, IRAN) and were used in accordance with the manufacturer's instructions.

Differential Growth medium based on the Edward-Ewing method included the following culture media; The main differential pipe was the TSI environment. Since Klebsiella pneumonia H2s-(A/A), after colony removal, the sample was transferred to the TSI from the Blood Agar medium, and then the incubation was carried out at 37°C for 24 hours. After observing the tube, Level and depth of yellow and the lack of hydrogen sulfide gas production, the next stage of the experiment began with the reaction (++-) IMViC (Indole, Methyl Red, Peppers and Citrate) and Urease Test MR-VP Growth medium; To identify the bacterial species, the bacteria cultured on the EMB medium were transferred to the MR-VP medium and incubated at 35°C for 24 hours. The VP test was carried out by adding reagents to 0.6 ml of alpha-naphthol 5% and then adding 0.2 ml of Potassium hydroxide (KOH) reagent and then the medium was placed in the vicinity of the air for 15 minutes. In order to carry out the Methyl red (MR) test, after 48 hours incubating the medium, 2.5 ml of agar broth was added to the medium, and then 5 drops of the methyl red specimen were added to the medium.

Urea agar base Growth medium; After bacterial transfer to the culture medium, it was incubated for 24 to 48 hours at 35°C, and the test was positive after 3 to 5 hours of protease reaction to create a pink color to confirm the presence of the microorganism.

Sim Growth medium; In this medium, the production of hydrogen sulfide gas -indole and motion are examined.

I) Production of black Hydrogen sulfide (SH2) gas. In this environment, there is an amino acid of cysteine. If the bacterium contains a cysteine enzyme, it consumes and produces amino acid cysteine, sulfur is present in the combined medium and produces SH2. II) The production of endodontic gas: In this amino acid environment, tryptophan is present if the bacterium has a tryptophanase enzyme.

Tryptophan converts into endodontic ally colored gas. If added to the environment, the coaxial reagents (ethyl alcohol) react with the endodontic gas in the environment and produce a purple ring (3).

Movement; If the bacterium is only on the path of cultivation Given, it grows motionless and if it grows and grows in general.

TSI Growth medium; The TSI environment contains phenol-reed, phenolic sulfate, sodium thiosulfate (to detect the production of hydrogen sulfide gas) and three glucose, lactose and sucrose sugars, with glucose concentrations in the range of 0.1 in two concentrations of bicarbonate sugar.

Molecular experiments

In order to design the primers used in this study, magA and rmpA2 sequences were taken from Klebsiella pneumonia strain K1 and K2, respectively, and the direct and inverse primer was used to propagate sequences belonging to the cps gene cluster. In order to design primers sequences received from the database access code format FASTA get web based software primer 3 plus Moved to reproduce Area 800 bp for gene magA and 600 bp for gene rmpA2 about design placement. The primer information used in Table 1 below is shown. Designed primers were also tested for performance in the oligocalc web application, which was synthesized in the South Korean company Bioneer.

In each reaction team, both primers were used to identify colony serotypes. The ratio of materials used and the thermal cycling used in the reaction are shown in Tables 2 and 3.

After polymerase chain amplification reaction, the reaction product was loaded at a rate of 5 µL on 3.1% agarose jelly. And using electrophoresis at 85 mA, voltage 70-volt breakdown took place after placing in ethidium bromide (0.5 mg/ml) for 10 minutes and rinse with distilled water for 5 minutes, using a UVITtech of The image gel was made (Figure 1).

Antibiotic Resistance Determination; In order to estimate the antibiotic resistance, isolates derived from the Clinical and Laboratory Standards Institute (CLSI) 2017 and using McFarland's half-concentration were cultured on Muller Hilton agar culture medium, and six antibiotic discs were cultured in each culture plate, which were made of OFX

antibiotics (OFX), Trimethoprim (TMP), ceftriaxone (CRO), Cefotaxime (CTX), cefazolin (CZ), and nitrofurantoin (FM) were used individually and after encoding at 37 °C for one day, formed halos Measured on the culture media using calcium. Determination of antibiotic resistance in all specimens was estimated using culture media on the Muller Hinton Agar medium using disc diffusion method based on CLSI standards of 2017. Antibiotic disks used by Thermofisher Oxide (ThermofisheroXid tm) manufactured in the United States.

RESULTS

The results obtained from electrophoresis show that 76% of the samples were K1 and 24% of the K2 serotype. Also, among the 35 samples collected from patients' urine, K1 serotype showed an 80% prevalence and among the samples belonging to the burn injuries, 71% had serotype K1 and 29% serotype K2 (Figure 1). In addition, serotype K1 showed a higher incidence than serum K2. Also, the results obtained with 6 antibiotics for each serotype showed a high resistance to Nitrofurantoin and Afloxacin antibiotics (Table 4, 5 and figure 2).

DISCUSSION

Klebsiella pneumonia is an opportunistic pathogen which is the main cause of nosocomial infections, in particular UTI, pneumonia, and blood infections. Most of the patients in the sample have burn injuries or infections. The urine was due to K1 and K2 serotypes. In the current study, the magA gene and rmpA2 encoding protein levels involved in the development of the disease were used to determine the serotype. Many previous studies have used genes such as wzi, wcaG and orf-10 to detect the serotypes (16-20). The results obtained by amplification of the PCR assay for replication of the magA gene for serotype K1 and rmpA2 for serotype K2 were validated for duplication of lengths of 800 and 600, respectively, and showed a high segment of primers designed to detect serotypes, which can be said to be a technique PCR can act as a useful and practical tool for identifying pathogenic bacteria based on molecular methods. In the study by Yeh et al., in 2006, Kelbisila pneumonia and populations based on the rmpA and magA genes in K1 and K2 serotypes in Singapore and Taiwan, based on the importance of the prevalence of this bacterial infection among patients and infections with the hospital's origin, which was performed on a population of 73, showed the presence of magA and rmpA genes using the PCR technique. It was estimated that the prevalence of serotypes K1 by forty-sixth and a half percent and K2 by 20 and a half percent in the total population The study found that magA and rmpA genes were two major genes in the production of serotype capsules and Can be used as a key factor in the identification of brigadier populations in population studies (12). In studies by Faizabadi et al., the detection of bridges K1 and K2 by using PCR technique on 89 individuals in Tehran in 2012 was also based on other genes involved in the cps gene cluster, such as wzc and orf10, and it was shown that of the total population studied Given, 16% belongs to serotype k1 and 11% belongs to serotype K2. It has been shown that this serotype has a high incidence of pathogenicity and PCR technique can act as a useful and practical tool for identifying pathogenic bacteria based on molecular methods (5). In a recent study conducted by Ononori et al., the identification of K. Pneumonia and its high prevalence was carried out using a general analysis of the genome in Italy. In this study, a single clone was responsible for the outbreak in the department. ICU, which was responsible for antibiotic resistance to penicillin, was identified. In this study, it has been shown that the use of molecular techniques, a combined phenotype technique, that the general sequence of the genome can help to quickly and accurately identify the pathogens of Klebsiella causing infectious diseases (19). Gierczynski et al. in 2007, Claimed that isolates of Klebsiella as one of the most important bacteria involved in the development of hospital-acquired infections, the PCR method was used. In a population survey of 147 individuals, among individuals. The findings revealed that 69% of the subjects had K1 serotype and 31% of the remaining K2 serotype, and the K1 serotype showed a much higher incidence. The genes in the CPS gene cluster among strains could be useful as well. Effective in detecting serotypes using molecular methods (9). The data obtained from this study also showed that the higher serotype K1 in comparison to K2 in isolates showed that the identified serotypes were also successful using the pcr molecular method. In a study by Zamani et al in 2013, a study of the identification of magA gene in medical isolates in Hamedan hospitals has been completed for 12 months, based on PCR-based identification, of which data show that among the 105 collected samples Using this technique, 95% of the samples were identified as Klebsiella pneumonia and magA gene was detected in 30% of samples (20). Also, the results of the antibiogram test showed that K1 serotype is resistant to the onloxacin and nitrofurantoin antibiotics and to ceftoxime and ceftriaxone antibiotics and has a moderate effect on trimethoprim and cefazolin antibiotics and the serotype K2 is antibiotics ceftoxime and ceftriaxone Susceptible to antibiotics that are resistant to nitrofurantoin and has a moderate effect on cefazolin, afloxacin and trimethoprim antibiotics. The percentage of investigated resistance in both serotypes shows a relatively higher resistance to serotype K1 than serotype K2. Sensitive antibiotics It is recommended to use The top ten include Cefotaxime and Ceftriaxone, and resistant antibiotics that should be used less than nitrofurantoin and afloxacin. In a study done by Manikandan and Amsath in 2013 to determine the antibiotic susceptibility of this bacterium, the results show a high sensitivity of 50% to the antibiotic nitrofurantoin and a 20% sensitivity to Ofloxacin and shows high antibiotics ceftriaxone, Cefotaxime and Cephazolin (14). In studies conducted by Vargheses et al in 2016, antibiotic susceptibility testing of Klebsiella pneumonia strains in the administration of anesthetic duct has also been resistant to 65 percent (15). Other Toroglu and Keskin studies performed in 2011 in the study of sensitivity and Resistance to pneumonia isolates from various infectious diseases in Turkey has been reported to be 77% resistant to nitrofurantoin antibiotics and 64% to Ophlocasin (16). In

examining the results of this study and comparing it with other reports, differences in some cases are quite evident, which can be due to the possibility of error during the test. Because of the impact of the correction of antibiotics used on serotypes and the identification of in vitro susceptible and susceptible forms, there are important points such as the type of antibiotic disks used and the variety in disc manufacturer's manufacturing companies, the depth and composition of the culture medium. It is possible that the resistance data will undergo false changes and errors in overall results. For example, the thinness of the culture medium will increase the sensitivity of antibiotics falsely. In principle, for the purpose of this type of testing, the depth of 4 mm is considered and the recommendation It turns out. In addition, the distribution and distribution of resistant strains as well as the different prevalence of serotypes in different regions can be due to climatic conditions, excessive consumption of antibiotics in that area (20-17).

CONCLUSION

In both serotypes, the relatively high resistance of K1 serotype to the serotype K2 is shown. Also, in both groups, the highest resistance to antibiotics is Nitrofenutin and onloxacin. In addition, the most commonly used antibiotics that are recommended are Cefotaxime and ceftriaxone, and resistant antibiotics that should be used less than nitrofurantoin and onloxacin. Proportion of resistances checked.

ACNOWLEDGMENTS (FUNDING SOURSE)

This article is outcome of the dissertation Miss Rezvan Doustipour, MSc student, Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.

AUTHOR CONTRIBUTIONS

All authors contributed in present study.

CONFLICT OF INTERESTS

There is no conflict of interest.

ETHICAL STANDARDS

All participants signed the written informed consent.

Table 1. Specifications for the primers used in this study

Primers name	percent GC	Primer sequence	length	T_m
magAF	57.00	GGTGCTCTTTACATCATTGC	20 b	54 ℃
magAR	56.00	GCAATGGCCATTTGCGTTAG	20 b	55 ℃
wzyF	55.55	GACCCGATATTCATACTTGACAGAG	26 b	52.5℃
wzyR	55.55	CCTGAAGTAAAATCGTAAATAGATGGC	28 b	54℃

Table 2. The composition of the materials used in each polyester chain replication reaction

material	Concentration in the base solution	Used volume
Nuclear free water	-	3.4
each primer	5 pmol/µl	2+2
Enzyme Tag DNA polymerase	10 µl	0.1
MgCl2	50 mM	0.2
dNTP	10 mM	0.3
Clooney sample as a replication template of the PCR buffer	0.9 µg/µl	1
	10 X	1
Total	-	10

Abbreviations;
$MgCl_2$: Magnesium chloride, dNTP: deoxynucleotides, PCR: polymerase chain reaction

Table 3. The thermal cycling used for each polymerase chain propagation reaction

Number of cycles	level	Time	Temperature
1	Primary Denaturing	5 min	94 ℃
32	Denaturing	35 sec	94 ℃
32	Connection	35 sec	55 ℃
32	Expansion	35 sec	72 ℃
1	Final Expansion	7 min	72 ℃

Table 4. Antibiogram obtained and their resistance or susceptibility to the samples observed in the serotype K1

Antibiotics	Resistance percent	Average observed	S	I	R
Ofx	89	13	17>	15	14<
Tmp	19	15	16>	11-15	10<
Cro	8	14/5	14-20>	-	13<
Ctx	7	16	15-19>	-	14<
Cz	21	15/5	-	15-17	14<
Fm	85	10	17>	15-16	14<

Abbreviations;
Ofx: Ofloxacin, Tmp: Trimethoprim, Cro: Ceftriaxone, Ctx: Cefotaxime, Cz: Cefazolin, Fm: Nitrofurantoin

Table 5. Antibiogram obtained and their resistance or susceptibility to the samples observed in the serotype K2

Antibiotics	Resistance percent	Average observed	S	I	R
Ofx	89	13	17>	15	14<
Tmp	19	15	16>	11-15	10<
Cro	8	14/5	14-20>	-	13<
Ctx	7	16	15-19>	-	14<
Cz	21	15/5	-	15-17	14<
Fm	85	10	17>	15-16	14<

Abbreviations;
Ofx: Ofloxacin, Tmp: Trimethoprim, Cro: Ceftriaxone, Ctx: Cefotaxime, Cz: Cefazolin, Fm: Nitrofurantoin

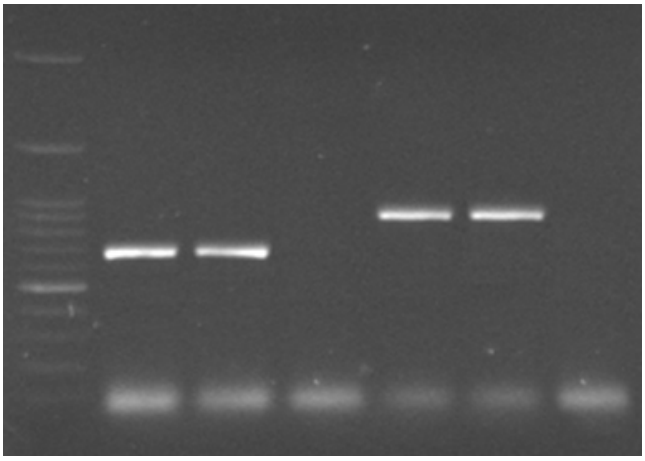

Figure 1. Band pattern obtained from PCR product (Ladder: marker, 1: fragment obtained for wzy gene, 2: positive control for wzr gene)

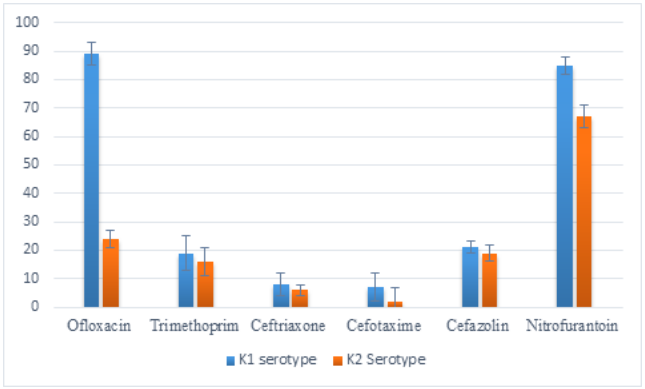

Figure 2. The degree of resistance tested in K1 and K2 serotypes in the use of different antibiotics

REFERENCES

1. Pan YJ, Lin TL, Chen YH, Hsu CR, Hsieh PF, Wu MC. Capsular types of Klebsiellapneumoniae revisited by wzc sequencing. PLoS One. 2013;8:78-8
2. Yeh KM, Lin JC, Yin FY, Fung CP, Hung HC, Siu LK, et al. Revisiting the importance of virulence determinant magA and its surrounding genes in Klebsiellapneumoniae causing pyogenic liver abscesses: exact role in serotype K1 capsule formation. J Infect Dis. 2010;201:1259-67
3. Lin JC, Koh TH, Lee N, Fung CP, Chang FY, Tsai YK. Genotypes and virulence in serotype K2 Klebsiella pneumoniae from liver abscess and non-infectious carriers in Hong Kong, Singapore and Taiwan. Gut pathogens J. 2014;6;1
4. Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods. Manual Clin Microbiol. 2015;8:1253-73
5. Stratchounski LS, Kozlov RS, Rechedko GK, Stetsiouk OU, Chavrikova EP. Antimicrobial resistance patterns among aerobic gram-negative bacilli isolated from patients in intensive care units: results of a multicenter study in Russia. J Clin microbial Infect; 1998;4:497-507
6. Chou HC, Lee CZ, Ma LC, Fang CT, Chang SC, Wang JT. Isolation of a chromosomal region of Klebsiellapneumoniae associated with allantoin metabolism and liver infection. Infect immun. 2004;72:3783-92

7. Horan T, Culver D, Jarvis W, Emori G, Banerjee S, Martone W, Thornsberry C. Pathogens causing nosocomial infections preliminary data from the national nosocomial infections surveillance system. Antimicrobic Newsletter. 1988;5:65-7
8. Siu LK, Fung CP, Chang FY, Lee N, Yeh KM, Koh TH. Molecular typing and virulence analysis of serotype K1 Klebsiella pneumoniae strains isolated from liver abscess patients and stool samples from noninfectious subjects in Hong Kong, Singapore, and Taiwan. J Clin Microbial. 2011;49:3761-5
9. Bencic I, Baudoin DV. Imipenem consumption and gram-negative pathogen resistance to imipenem at Sestre Milosrdnice University Hospital. Actaclincroat J. 2001; 40:185-9
10. Mshana SE, Kamugisha E, Mirambo M, Chakraborty T. Prevalence of multi resistant gram-negative organisms in a tertiary hospital in Mwanza, Tanzania. BMC Res Notes. 2009;2:49-55
11. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin microbiol rev.1998;11:589-603
12. Yeh KM, Kurup A, Siu LK, Koh YL, Fung CP. Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for Klebsiellapneumoniae liver abscess in Singapore and Taiwan. J Clin Microbial. 2007;45:466-71
13. Soltan Dalal MM, Miremadi SA, Sharify Yazdi MK, RastegarLari A, Rajabi Z, AvadisYans S. Antimicrobial resistance trends of Klebsiella Spp. isolated from patients in Imam Khomeini hospital. J Payavard Salamat. 2012;6:275-81
14. Manikandan C, Amsath A. Antibiotic susceptibility of bacterial strains isolated from patients with respiratory tract infections. IJPAZ. 2013;1:61-9
15. Varghese A, George S, Radhakuttyamma Gopalakrishnan AM. Antibiotic susceptibility pattern of klebsiella pneumonia pneumonaite isolated from cases of urinary tract infection in a tertiary care setup. JEMDS. 2016;5:1470-4
16. Toroglu S, Keskin D. Antimicrobial Resistance and Sensitivity among Isolates of Klebsiellapneumoniae from Hospital Patients in Turkey. Int J Agric Biol. 2011;13:61-70
17. Barreto S, Zambrano M, Araque M. Phenotypic variations of susceptibility in Klebsiellapneumoniae strains of nosocomial origin and their association with biofilm formation. Inves Clin. 2009;50:221-9
18. Schroll C, Barken KB, Krogfelt KA, Struve C. Role of type 1 and type 3 fimbriae in Klebsiellapneumoniae biofilm formation. BMC microbiol. 2010;10:1- 10
19. Wiskur BJ, Hunt JJ, Callegan MC. Hypermucoviscosity as a virulence factor in experimental Klebsiella pneumonia endophthalmitis. Invest Ophthalmol Vis Sci. 2008;49:4931-8
20. Karbasizaed V, Badami N, Emtiazi G.Antimicrobial, heavy metal resistance and plasmid profile of coliforms isolated from nosocomial infections in a hospital in Isfahan, Iran. Afr J Biotechnol. 2003;2:379-83