Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(11):326-331

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antimicrobial sensitivity pattern of *Klebsiella pneumonia* isolated from pus from tertiary care hospital and issues related to the rational selection of antimicrobials

Asati Rakesh Kumar

Department of Pharmacology, Peoples College of Medical Science & Research Centre and Peoples University, Bhanpur, Bhopal, India

---

ABSTRACT

Antimicrobial resistance is not only increasing the healthcare costs but also the severity and death rates from certain infections that could have been avoided by prudent and rational use of the existing and newer antimicrobial agents. Prudent and rational use of antimicrobial is possible by forming local, national and global wide antibiogram. The present study is undertaken to prepare local antibiogram of Klebsiella pneumoniae (K. pneumoniae) isolated from pus and to discuss general issues related to antimicrobials use. Total 198 pus samples were processed for culture sensitivity testing. Out of 198 pus samples, 142 organisms were isolated. Staphylococcus aureus (36.7%) accounted most common organism and K. pneumoniae(21.1 %) accounted 2nd most common organism. Other organisms were Pseudomonas spp. (18.3 %), Escherichia coli (12.7 %), streptococci aureus (9.8 %) and Proteus spp. (1.4 %).Antimicrobial susceptibility testing was done by disk diffusion method described by Kirby-Bauer (1961). K. pneumonia was found to be most sensitive to amikacin, gatifloxacin, gentamicin and chloramphenicol. Consequently, considering the antimicrobial susceptibility, cost, side effects and many other factors, amikacin, gatifloxacin, gentamicin and chloramphenicol should be preferred drugs for K. pneumoniae infection isolated from pus.

Keywords: *K. pneumoniae*, antibiotic susceptibility testing, antimicrobial resistance, rational selection of antimicrobials.

INTRODUCTION

In recent years, drug resistance to human pathogenic bacteria is being commonly reported from all over the world [1, 2]. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics [1, 3] although pharmacological industries have produced large number of newer antibiotics in the last three decades. Reason behind this is that microorganisms are becoming resistant to both older and newer antibiotics. In addition, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents and transferring the resistance from one bacteria to other [1,4]. Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual era dictation of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms [1, 5].

The antimicrobial agents are of great value for devising curative measures against bacterial infections. The indiscriminate use of antibiotics has led to the emergence of antimicrobial resistance in various isolates of bacteria. Resistant bacteria impacts the public health in such a way that it increases morbidity and mortality from treatment failures and increases healthcare costs as newer and more expensive antibiotics are needed to treat infections [6].

Resistant bacteria are emerging worldwide as a threat to the favourable outcome of common infections in community and hospital settings. Staphylococcus aureus showed resistant due to the production of penicillinase with the ability to hydrolyzing penicillin; first generation resistant due to β-lactamases and third-generation cephalosporins are resistant due to the production of extended-spectrum β-lactamases (ESBLs) [7].

Microorganisms are the concealed enemies to the mankind and cause a very profound damage in human body as well as other living organism. The agents, which have the capacity to kill the microbes or arrest the multiplication, are called the antimicrobial agents or drugs. There are a lot of antimicrobial drugs of which some are discovered or established [8].

Klebsiella pneumoniae (K. pneumoniae) are ubiquitously present and reported worldwide. In recent years, *K. pneumonia* have become important pathogens in nosocomial infections[9]. The importance of *K. pneumoniae* in the ever increasing number of gram negative aerobic bacillary nosocomial infections in the United States [10] and India [11] has been well documented. Epidemic and endemic nosocomial infections caused by *K. pneumonia* species are leading causes of morbidity and mortality [12].Recently, World Health Organization also warned the community that multidrug resistant bacteria are emerging worldwide which is a big challenge to healthcare. If we don't take immediate action then only handful antibiotics will be left to cure diseases [13].Multidrug resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate by using available antibiotics. Moreover, extensive use of broad-spectrum antibiotics in hospitalized patients has led to increased prevalence of *K. Pneumonia* as well as development of multidrug resistant strains of *K. pneumoniae*.

The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistant pathogens, fueling an ever-increasing need for new drugs. However, the pace of antimicrobial drug development has slowed dramatically, with only a handful of new agents, few of which are novel, been introduced into clinical practice each year. Reducing the inappropriate antibiotic use is thought to be the best way to control resistance [14].

The microbiology laboratory plays a central role in the decision to choose a particular antimicrobial agent over others. First, causative organism is identified and isolated when the patients' specimens are sent to the microbiology laboratory. Once the microbial species causing the disease have been identified, a rational choice of the class of antibiotics likely to work in on the patient can be made [15].

The aim and objectives of the present study was to find out the prevalence and antimicrobial susceptibility of *K. pneumoniae* isolated from pus samples and to discuss issue related to rational selection of antimicrobials in Surendranagar, Gujarat area.

EXPERIMENTAL SECTION

In the present study, 198 pus samples were collected in Department of Microbiology from inpatient & outpatient department of C.U. Shah Medical College & Hospital Surendranagar from period Jan 2008 to December 2008. First step done was to isolate the organisms from these pus samples and then to study the culture susceptibility in *Klebsiella pneumoniae (K. pneumoniae).*

The samples were inoculated on blood agar and mac conkey agar and incubated overnight at 37ºC.

K. pneumoniae strains were identified using morphological, microscopy and biochemical tests following standard procedures described by Cowan and Steel (1974) and Cheesborough (2006) [16, 17]. Morphology of *K. pneumoniae* identified were large, dome-shaped, mucoid colonies on blood agar and lactose fermenting colonies on Mac conkey agar. In Gram-staining, gram-negative, short, plump, straight rods were seen. The biochemical characters identified were negative indole test, negative methyl red test, positive voges-proskauer test, positive citrate utilization test,

positive urease test, acid plus abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation test. Besides these tests, motility and growth of organism in potassium cyanide were also checked.

Antibiotic sensitivity test was performed by Kirby Bauer Disc Diffusion method [18]. A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates. Filter paper disks containing designated amount of the antimicrobial drugs obtained from commercial supply firms (Himedia Labs, Mumbai, India) were used. The Mueller Hinton agar plates were allowed to dry before applying antibiotic disc. Then same commercially available antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. If an antimicrobial activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter at 24 hours using a scale. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were considered as sensitive in the assessment of the data [19].

Antimicrobial susceptibility of *K. pneumoniae* to different antibiotics is obtained. From antimicrobial susceptibility, antibiogram for *K. pneumoniae* is prepared and consequently *c*onsidering the antimicrobial susceptibility, cost, side effects and many other factors probable drug of choice is selected for *K. pneumoniae*.

RESULTS

Table 1 Number and percentage of organisms isolated from pus samples

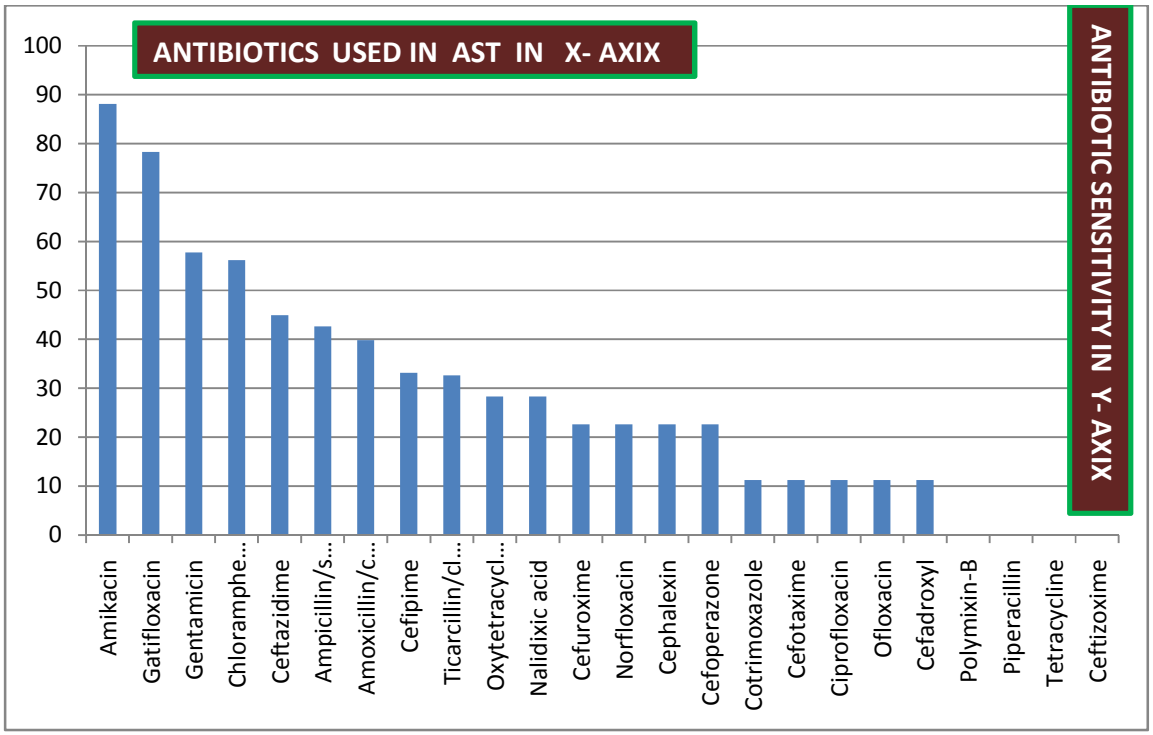
Serial number	Name of organism	Number of organism (n= 142)	Percentage of total organisms
1	*Staphylococcus aureus*	52	36.7 %
2	*Klebsiella pneumoniae*	30	21.1 %
3	*Pseudomonas spp.*	26	18.3 %
4	*Escherichia coli*	18	12.7 %
5	*streptococci aureus*	14	09.8 %
6	*Proteus spp.*	02	01.4 %
	Total	142	100 %

During the 12 month period, a total of 198 pus samples were processed for culture and sensitivity testing. Pus samples of patients of all age groups (1day-85years) and both sexes were processed. A total 142 different organisms were isolated from 198 pus samples thus culture positivity was 71.7 % as shown in Table-1.*Staphylococcus aureus*(36.7%) accounted most common organism. *Klebsiella pneumoniae* (21.1%) accounted 2nd most common organism. Other organisms isolated were *Pseudomonasspp.* (18.3 %), *Escherichia coli and* (12.7 %) *Streptococci spp. (9.8 %) and Proteus spp.* (1.4 %).

Table 2 Antibiotic sensitivity of *K. pneumoniae* isolated from pus samples

Antibiotics	Sensitivity in %	Resistance in %	Antibiotics	Sensitivity in %	Resistance in %
Amikacin	88.1	11.9	Norfloxacin	22.6	77.4
Gatifloxacin	78.3	21.7	Cephalexin	22.6	77.4
Gentamicin	57.8	42.2	Cefoperazone	22.6	77.4
Chloramphenicol	56.2	43.7	Cotrimoxazole	11.2	88.8
Ceftazidime	44.9	45.1	Cefotaxime	11.2	88.8
Ampicillin/sulb.	42.6	57.4	Ciprofloxacin	11.2	88.8
Amoxicillin/clav.	39.8	60.2	Ofloxacin	11.2	88.8
Cefipime	33.2	66.8	Cefadroxyl	11.2	88.8
Ticarcillin/clav.	32.6	67.4	Polymixin-B	0	100
Oxytetracycyline	28.3	71.7	Piperacillin	0	100
Nalidixic acid	28.3	71.7	Tetracycline	0	100
Cefuroxime	22.6	77.4	Ceftizoxime	0	100

Figure 1-Antibiotic sensitivity of *K. pneumoniae* isolated from pus samples



In present study, it has been demonstrated that *K. pneumonia* are showing significant amount of antimicrobials resistance to different antibiotics (Figure 1 and Table 2). Out of 24 antimicrobials tested for AST, *K. pneumoniae* is showing more than 50 % sensitivity only for four antimicrobials namely amikacin (88.1), gatifloxacin (77.8 %), gentamicin (57.8 %) and chloramphenicol (55.6 %). More than 20 antimicrobials (out of 24 antimicrobials tested)are showing less than 50 % antibiotic sensitivity. *K. pneumoniae* isdemonstrating 88.8% resistance to cotrimoxazole, cefotaxime, ciprofloxacin, ofloxacin and cefadroxyl and 100 % resistance to polymixin-b, piperacillin, tetracycline and ceftizoxime.

Table 3 Probable drug of choice for *K. pneumoniae* isolated from pus samples

Sr.no.	Name of drug	Sensi.	Route of Adm.	Price in Rs Per 10 tab /per vial	Dose and Total duration of treatment	Total cost For treatment in Rs	ADR/ Toxicity of drug
1	Amikacin	88.9 %	IV/IM	10/500 mg vial	15mg/kg in 3 divided doses for 5 days	90-100	Mild –mod.
2	Gatifloxacin	77.8 %	Oral/ IV	50	500 mg OD ×7-10 days	35-50	Mild
3	Gentamicin	57.8 %	IV/IM	80 mg x 2ml (8 INR)	180-300 mg in div. doses 8 hrly for 7-10 days	168-240	Mild
4	Chloramphenicol	55.6 %	Oral/iv	40	500 mg QID×7-10 days	150-160	Mod-severe

Abbr.- IV- Intravenous, IM-Intramuscular, Adm.- Administration, Sensi.- Sensitivity, ADR- Adverse Drug Reaction

Percentage antibiotic sensitivity of above 4 drugs is between 55.6 - 88.9 % (as shown in table-3), taking consideration of cost, adverse drug reaction and other factors; gatifloxacin should be preferred drug of choice. Alternatives can be amikacin, gentamicin and chloramphenicol.

DISCUSSION

Present study highlights the most alarming situation of highly diverse antibiotics resistance. In present study; K. *pneumoniae* demonstrates great extent of resistance to β lactam antibiotics, fluoroquinolones (except gatifloxacin), tetracyclines and cotrimoxazole. *K. pneumoniae* showed100 % resistance to polymixin-B, piperacillin, tetracycline

and ceftizoxime. Excessive and overuse of cephalosporin, quinolone, cotrimoxazole and tetracyclines antibiotics lead to emergence of resistance towards *K. pneumoniae*.

No doubt this represents very critical situation as compared to investigations from other regions of the world reporting resistance towards first, second and third generation cephalosporins [20]and other group of antibiotics.There are reports covering high levels of resistance of *K. pneumoniae* towards these antibiotics in other countries also [21]. This may be due to the production of β-lactamase enzymes which cause the hydrolysis of β-lactam ring resulting in inactivation of β-lactam antibiotics. Alarming finding seen in this study was that resistance shown to various third generation cephalosporins. Overall resistance to third generation cephalosporins was high on account of the production of extended spectrum β-lactamases (ESBLs) by the *K. pneumoniae*. The resistance may also be due to the production of metallo-β-lactamases (MBL), which can be chromosomally encoded or plasmid mediated. The dose as well as the incidence of toxicity subsequently reduced if beta lactamase inhibitors are used with β-lactam antibiotics[22]. Another mechanism is associated with penicillin-binding protein 2a (PBP2a), encoded by $mecA_2$. Another gene involved in penicillin resistance in staphylococci is blaZ which encodes β-lactamase$_2$[23].
In present study, *K. pneumoniae* showed least resistance to amikacin and gentamycin. These drugs are proposed to be an alternative and better treatment of *K. pneumoniae* infection in this part of the country. Furthermore, sensitivity of *K. pneumoniae* to amikacin and gentamycin could mean that there is a possibility of sensitivity to other aminoglycosides such as streptomycin, neomycin and kanamycin [24].

According to WHO/INRUD indicators, the average drug per prescription was high. Although, most of the drugs prescribed were from essential drug list, prescribing by generic name needs to be improved in our hospital to prevent medication errors and further adverse effects due to it. A regional antibiotic policy would further improve the prescription practices in our hospital. The policy could be established and monitored by a regional paediatric pharmacovigilance centre in our Hospital. This view was also expressed in another study conducted in the same hospital [25].

Antibiotic overuse, prescription of drugs with lack of proper sensitivity test and over dosing may have created this problem in developing nations. Multidrug resistance and the presence of several virulence factors in the strains of many pathogens responsible for different diseases pose an increasing threat to the successful management of disease course[26].

Because antimicrobial resistance patterns are continually evolving and multi-drug resistant (MDR) organisms undergo progressive antimicrobial resistance, continuously updated data on antimicrobial susceptibility profiles is essential to ensure the provision of safe and effective empiric therapies[27].

CONCLUSION

In summary, high antibiotic resistance of *K. pneumoniae* towards commonly used antibiotics are the major reasons for prolonged infections, increased hospitalization, increased cost of therapy and enhanced morbidity & mortality rates. Special care must be taken regarding treatment of infections especially in developing countries like India. Moreover physician must change their prescription priorities towards alternative treatments in management of infections specially *Klebsiella* spp.

The findings in present the study suggest that there is an urgent need for constant monitoring of susceptibility of pathogens in different populations to commonly used antimicrobial agents. The data of this study may be used to determine trends in antimicrobial susceptibilities, to formulate local antibiotic policies and overall to assist clinicians in the rational choice of antibiotic therapy to prevent misuse, or overuse, of antibiotics.

K. pneumoniae was found to be most sensitive to amikacin, gatifloxacin, gentamicin and chloramphenicol. Considering the antimicrobial susceptibility, cost, side effects and many other factors; amikacin, gatifloxacin, gentamicin and chloramphenicol should be preferred drugs for *K. pneumoniae* infection isolated from pus.

Acknowledgements
I am thankful to all the consultants of Department of Microbiology of C.U.Shah Medical College and hospital, Surendranagar who supported and guided in this work. I thank Dean, C.U.Shah Medical College and hospital, Surendranagar and Head of the Department of Microbiology and Pharmacology, C.U.Shah Medical College and

hospital, Surendranagar for their kind support in this work. Special thanks are given to Dr Sanjay Mehta, professor and Dr Kunjan Kikani, Associate Professor, Department of Microbiology, C.U.Shah Medical College for his timely help and encouragement during this work.

REFERENCES

[1] FirdausJahan, Rubina Lawrence, Vinod Kumar and Mohd. Junaid ,*Journal of Chemical and Pharmaceutical Research*, **2011,** 3(4):777-789
[2] KJVPiddock, R Wise. *J. Antimicrob. Chemother*.**1989** , 23, 475-483.
[3] I Ahmad, AZ Beg. *J. Ethnopharmacol* , **2001** , 74, 113-123.
[4] ML Cohen. *Science* .**1992** , 257, 1050-1055.
[5] H Harbottle, S Thakur, S Zhao, DG White. *Anim. Biotechnol*. **2006** , 17, 111-124.
[6] B.Srinu, A.Vijaya Kumar, E.Kumar, and T.MadhavaRao, *Journal of Chemical and Pharmaceutical Research*, **2012,** 4(7): 3734-3736.
[7] QuaserZafar Beg; AwdahMasoud Al-hazimi; Mohammed Qumani Ahmed; Mohammad Feroze Fazaludeen and RobinaShaheen; *Journal of Chemical and Pharmaceutical Research*,**2011,** 3(6):715-724.
[8] .S.Suganya., R.Bharathidasan, G.Senthilkumar.,P.Madhanraj and A. Panneerselvam. *Journal of Chemical and Pharmaceutical Research*, **2012,** 4(3):1846-1850
[10] J.R. Graybill, L. W. Marshall, P. Charache, C.K.Wallace and V.K.Melwin,*Am.Rev.Respir.Dis.* **1973**, 108:1130-1140.
[11] N.B. Mathur, A. Khalib,R. Sarkar and R.K. Puri, *Ind. J. Pediatri*.**1991**, 28(ii) 1259-1264.
[12] S.J. Cryz, R.Furer and R. Germanier., *Infect. Immun*.**1985,**45:139-142.
[13] S.YoungSoo , WHO, Western Pacific region, press release , **7 April 2011**.
[14]Henry Chambers F., Goodman & Gillman's, The Pharmacological basis of Therapeutics,11th edition,McGraw-Hill; New York,**2006**; p.1095.
[15] Goodman & Gillman's, The Pharmacological basis of Therapeutics, 12th edition, McGraw-Hill; New York ;**2010**; p-1369.
[16]M. Cheesborough.; Medical Laboratory Manual for Tropical Countries, II Edi. Microbiology (ELBS), New York, **2009**;p-62-70.
[17] S.J.Cowan and K.J.Steel; Cowan and Steel manual for identification of medical bacteria, 2nd ed. Cambridge University Press, London; **1974**.
[18] AW Bauer, WMM. Kirby, JC Sherris, M.Tuck, *Am J Clin Pathol* **1966,** 45: 493-6.
[19] C.Manikandan and A. Amsath, *Int. J. Pure Appl. Zool*., **2013** 1(1): 61-69.
[20]B. E.Murray, J. J. Methewson , H. L.Dupont, C. D. Ericsson, and R. R. Reves, *Antimicrobial Agents and Chemotherapy*, **1990**, 34, 515-518.
[21] A.Subha, and S. Ananthan, *Indian Journal of Medical Microbiology* **2002**, 20, 92-95.
[22]L.David Patersonand A.RobertBonomo, *ClinMicrobiol Rev*. **2005** October; 18(4): 657–686.
[23]Nizami Duran, BurcinOzer, GulayGulbol Duran, Yusuf Onlen&CemilDemir., *Indian J Med Res* 135, March **2012**, pp 389-396
[24]LD Kitara,1 AD Anywar, D Acullu, E Odongo-Aginya, J Aloyo, and M Fendu,*Afr Health Sci*. **2011** August; 11(Suppl 1): S34–S39.
[25]SushmaMuraraiah, AsthaSarda, Ayesha Romana and C R Jayanthi, *Journal of Chemical and Pharmaceutical Research*, **2012,** 4(6):3201-3206
[26]I.O. Okonko, E.A.Fajobi, T.A. Ogunnusi, A.A. Ogunjobi and C.H.Obiogbolu, *African Journal of Biomedical Research*, **2008,**Vol. 11; 235 – 250.
[27] A. O. Nkang, I. O. Okonko, O. K. Mejeha, O. G. Adewale, A. O. Udeze, A. Fowotade, E. A. Fajobi, A. O. Adedeji and E. T. Babalola. , *Journal of Microbiology and Antimicrobials*, **2009**, Vol. 1(2).pp. 019-026, November.