

Original Research Article

Isolation and antibiotic sensitivity of *Klebsiella pneumoniae* from pus, sputum and urine samples

K.N.Ravichitra^{1*}, P.Hema Prakash², S. Subbarayudu³ and U. Sreenivasa Rao⁴

Department of Microbiology, Alluri Sitarama Raju Academy of Medical Sciences (ASRAMS),
Eluru - 534 005 West Godavari District, Andhra Pradesh, India

*Corresponding author

A B S T R A C T

Keywords

Klebsiella pneumoniae;
nosocomial infections;
antimicrobial drug resistance;
ESBLs.

Gram negative pathogens are an important cause of community and hospital acquired infections throughout the world. *Klebsiella pneumoniae* has become one of the more common cause of these infections and one of the important aspects of *Klebsiella* associated infections is the emergence of multi-drug resistant strains particularly those involved in nosocomial diseases. So the knowledge of the resistivity pattern of *Klebsiella* isolates has been the global necessity. This study was done to determine the isolation rate of *Klebsiella*, their antibiogram and for the presence of resistant strains from various clinical samples. A total number of 698 pus, 312 sputum and 2176 urine samples were included in the present study. Isolates of *Klebsiella* were identified by standard microbiological techniques and their antibiogram determined by Kirby-Bauer disc diffusion method. Of the 3186 samples processed 1798 (56.4%) samples were culture positive for various organisms. A total of 1871 organisms were isolated, of them 480 (25.6%) were *Klebsiella pneumoniae*. The frequency of ESBL producers in our study was 76 (15.8%) of all *Klebsiella* isolates. The present study reveals the frequency of isolation of *Klebsiella* from various clinical samples and their tendency towards antibiotic resistance.

Introduction

In 1883 Friedlander isolated a capsulated bacillus from the lungs of patients who had died of pneumonia (Patrick R Murray, 2005). This was named after him as Friedlander's bacillus. Later on this organism was given the generic name of *Klebsiella*, which is ubiquitously present and reported worldwide.

Klebsiella is a gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family (Elmer W. Koneman, 2006). It is the second most popular member of the aerobic bacterial flora of the human intestine. It is the most common causative agent of nosocomial and community acquired infections.

It has even replaced *Escherichia coli* in some centers as a nosocomial pathogen. It causes pneumonia, urinary tract infection, other pyogenic infections, septicemia and rarely diarrhea (Arti Kapil, 2013).

Biochemically typical strains of *Klebsiella pneumoniae* are resistant to a wider range of antibiotics than are most *Escherichia coli* strains. They are nearly always naturally resistant to ampicillin (Patrick R Murray, 2005). Resistance of *Klebsiella* to previously sensitive antibiotics is also increasing in the recent years due to overuse and misuse of antimicrobial agents and or natural causes.

Of particular concern is the Extended Spectrum Beta Lactamase (ESBL) producing *Klebsiella pneumoniae* that have been steadily increasing over the past years and rapidly spreading worldwide that pose a serious threat for healthcare associated infections. Increasingly the ESBL *Klebsiella pneumoniae* are also showing co-resistance to other antimicrobial agents like quinolones and aminoglycoside antibiotics. Both morbidity and mortality is increased when infection is caused by these drug resistant organisms. Antibiotic sensitivity pattern may change from time to time and place to place.

Therefore updated knowledge of the drug resistance pattern in a particular region is useful in clinical practice. This work gives an account of isolation of *Klebsiella* from clinical pus, sputum and urine samples, their antibiogram and presence of resistant strains in various samples.

Materials and Methods

Collection of samples

This study was carried from November 2012 to October 2013 at the Department of

Microbiology. A total of 698 pus, 312 sputum and 2176 urine samples were collected during this period with universal safety precautions and were transported to the laboratory without delay. The pus samples were either aspirated by disposable syringes or collected onto sterile cotton tipped swabs. Sputum and clean voided midstream urine was collected into screw top containers (Patrica M. Tille, 2014). Samples were obtained from both inpatients and outpatients, of all age groups and of both sexes.

Characterization of bacterial isolates

Pus, sputum and urine samples were aseptically inoculated on to Blood and MacConkey agar plates and incubated overnight at 37°C. *Klebsiella* isolates were identified by their morphology and biochemical characteristics. Morphology of *Klebsiella* identified were large dome shaped colonies on blood agar and lactose fermenting mucoid colonies on MacConkey agar. In gram staining, gram negative, short, plump, straight rods were seen. The biochemical characters identified were positive Voges-Proskauer test, positive citrate utilization test, positive urease test, acid and abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests (Elmer W. Koneman, 2006; J. Gerald collee, 2012).

Antimicrobial susceptibility testing

Was done for all the isolates on Mueller-Hinton agar plates by Kirby-Bauer disc diffusion method according to the CLSI guidelines 2012 (CLSI, 2012). Reduced susceptibility to cefotaxime (30 µg) and Ceftriaxone (30 µg) with zone sizes $\leq 27\text{mm}$ and $\leq 25\text{mm}$ respectively were used as screening method for ESBL production.

Results and Discussion

A total of 3186 pus, sputum and urine samples were processed during our study period. 1871 bacterial isolates were obtained from culture positive 1798 samples. Of them 480 were *Klebsiella pneumoniae* (Table -1 & chart-1). The sensitivity and resistance pattern of the *Klebsiella* isolates to various antibiotics in our study is shown in the table -2. Of the 480 *Klebsiella* isolated, 76 (15.8%) were ESBL producers, 36 isolates were from pus samples, constituting 23.1% of all pus isolates, 15 were from sputum samples constituting 22.1% of all sputum isolates and 25 were from urine samples constituting 9.8% of all urine samples.

Klebsiella species have been associated with different types of infections. However the main importance of *Klebsiella* as a pathogen is in causing infections in hospitalized patients, the strains responsible are nearly always biochemically typical members of *Klebsiella pneumoniae* (Patrick R Murray, 2005).

Moreover, extensive use of broad spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and the development of multidrug resistant strains like those of Extended Spectrum Beta Lactamases (ESBLs) (Archana Singh Sikarwar et al., 2011). These multidrug resistant strains cause serious nosocomial and community acquired infections that are hard to eradicate by using available antibiotics. Hence the need to determine the antibiogram of these pathogens in order to evaluate the efficiency of empirical drug treatments formulated in our hospitals.

In the present study culture positivity for *Klebsiella* in sputum was 30.9% which is

in good agreement with Manikandan et al (2013) followed by 29.2% in pus samples, which is similar to Valarmathi et al (2013) and 22.9% in urine samples which is in agreement with R.Sarath babu et al (2012). Most of the isolates in our study were not multidrug resistant. The frequency of ESBL producers in our study was 76 (15.8%) of all *Klebsiella* isolates. Of them isolates from pus constituted the highest 36 (23.1%) of all pus isolates, which is in good agreement with reports from B.L.Chaudhary et al (2013), followed by 10 (14.7%) of sputum isolates which is little higher than that reported by Ugur Gonlugur et al (2004) and 30 (11.7%) of all urine isolates which is similar to Shamweel Ahmed et al (2009). Our study showed good sensitivity to imipenem, accounting for 94.9% of pus, 80.4% of sputum and 89.9% of urine samples. Sensitivity to cefaperazone and sulbactam was also good accounting to 81.1% of pus, 88.2% of sputum and 89.1% of urine samples. Sensitivity to quinolones and aminoglycosides antibiotics was moderate in our study.

The present study reveals the incidence of infections due to *Klebsiella* and their tendency towards antibiotic resistance. Multidrug resistant bacteria are emerging worldwide which causes major public health problems and challenges to health care.

Knowledge about the common organisms associated with infections, the resistance patterns of these bacterial strains in a geographical area will help to guide appropriate and judicious antibiotic use, formulate antibiotic policies and for infection control intervention programmes. However only screening tests were performed for detection of ESBLs in our study, confirmatory studies are required for further evaluation.

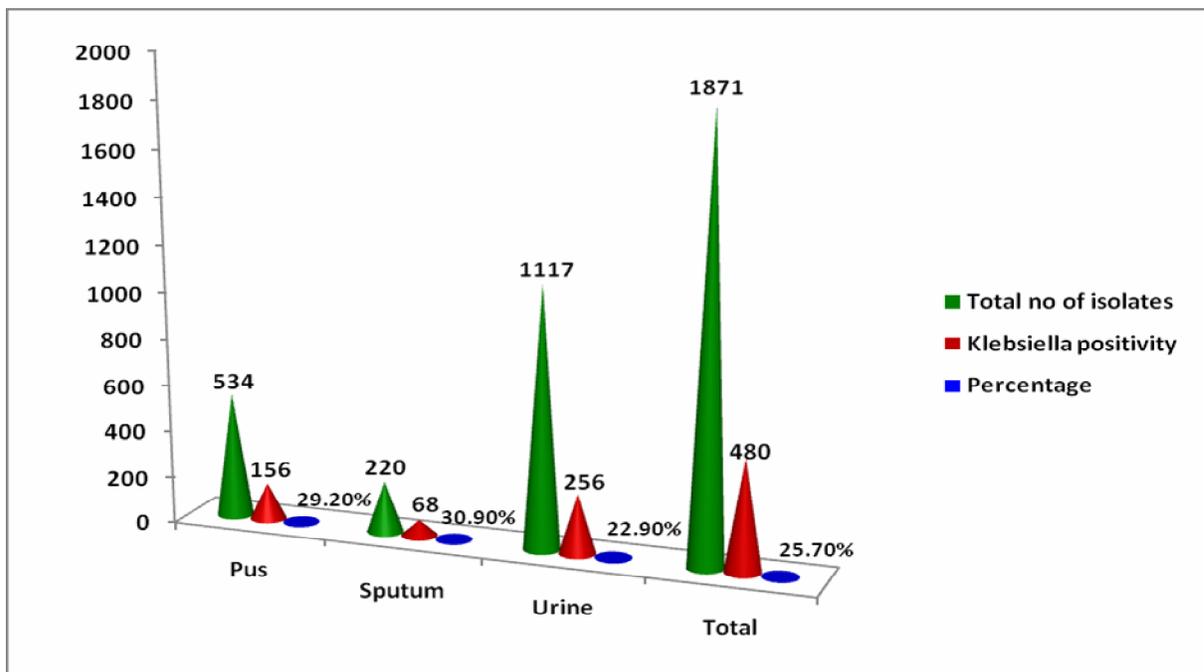
Table.1 Total number of organisms isolated

Sample	Total no of samples	Number of Culture positives	% of culture positives
Pus	698	482	69.1
Sputum	312	212	67.9
Urine	2176	1104	50.7
Total	3186	1798	56.4

Table.2 Antibiotic sensitivity pattern

Antibiotics	Pus		Sputum		Urine	
	R %	S%	R%	S%	R%	S%
Amoxyclav	58.2	41.8	78.4	21.6	65.9	34.1
Ofloxacin	35.4	64.6	33.3	66.7	40.3	59.7
Cefotaxime	23.1	76.9	19.8	80.2	8.7	91.3
Ceftriaxone	20.6	79.4	22.1	77.9	9.8	90.2
Gentamicin	12.7	87.3	37.6	62.4	40.3	59.7
Cefoperazone& sulbactam	18.9	81.1	11.8	88.2	10.9	89.1
Imipenem	5.1	94.9	19.6	80.4	10.1	89.9

Chart.1 Culture positivity of *Klebsiella pneumoniae*



Acknowledgement

I thank the management of ASRAM Medical College and HOD of Microbiology Department, Dr. S. Subbarayudu for allowing me to conduct this short study.

References

- Archana Singh Sikarwar and Harsh Vardhan Batra. 2011. Prevalence of Antimicrobial Drug Resistance of *Klebsiella pneumoniae* in India. *International Journal of Bioscience, Biochemistry and Bioinformatics*. 1(3): 211-215.
- Arti Kapil. 2013. Ananthanarayan & Paniker's Textbook of Microbiology. 9th edition. Chennai: Universities Press Pvt Ltd.
- Chaudhary, B.L., Anahita, V. Bhesania Hodiwala, S. A. Samant, Rakesh Kumar Mukhia, Rakesh Prasad and A. D. Urhekar. 2013. Detection of Extended Spectrum β - Lactamase & Amp C in Clinical Isolates of *Klebsiella* Species. *GRA - Global Research Analysis*. 2(8): 154-156.
- Elmer W. Koneman, Stephen D. Allen, William M. Janda, Paul C. Schreckenberger and Washington C. Winn. 2006. *Color Atlas And Textbook Of Diagnostic Microbiology*. 6th edition. Baltimore : Lippincott Williams Wilkins.
- J.Gerald Collee, Andrew G.Fraser, Barrie P. Marmion and Anthony Simmons. 2012. *Mackie & Mc Cartney: Practical Medical Microbiology*. 14th edition. Churchill Livingstone: Elsevier.
- Manikandan, C., and A.Amsath. 2013. Antibiotic susceptibility of bacterial strains isolated from patients with respiratory tract infections. *International Journal of Pure and Applied Zoology*. 1(1): 61-69.
- Patricia M. Tille. 2014. *Bailey & Scott's Diagnostic Microbiology*. 13th edition. St. Louis, Missouri : Mosby Elsevier publications.
- Patrick R Murray, Barry Holmes, Hazel M. Aucken. 2005. *Topley & Wilson's Microbiology & Microbial Infections*. Volume 2. 10th edition. Salisbury, UK: Edward Arnold Ltd.
- Sarathbabu, R., T.V.Ramani, K.Bhaskara rao and Supriya Panda. 2012. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples. *IOSR Journal of Pharmacy and Biological Sciences (IOSRJPBS)*. 1(2): 04-09.
- Shamweel Ahmad, Nami Fawazan Al-Juaid, Faris Q. Alenzi, Essam H. Mattar and Osama El-Sayed Bakheet. 2009. Prevalence, Antibiotic Susceptibility Pattern and Production of Extended-Spectrum - β Lactamases Amongst Clinical Isolates of *Klebsiella pneumoniae* at Armed Forces Hospital in Saudi Arabia. *Journal of the College of Physicians and Surgeons Pakistan*. 19(4): 264-265.
- Ugur Gonlugur, Mustafa Zahir Bakici, Ibrahim Akkurt and Tanseli Efeoglu. 2004. Antibiotic susceptibility patterns among respiratory isolates of Gram negative bacilli in a Turkish university hospital. *BMC Microbiology*. 4(32).
- Valarmathi, S., M. Rajasekara Pandian and B. Senthilkumar. 2013. Incidence and screening of wound infection causing microorganisms. *J. Acad. Indus. Res.* 1(8): 508.
- Wayne, Pennsylvania 19087 USA. Clinical Laboratory Standards Institute. 2012. Performance Standards for Antimicrobial Disc Susceptibility Tests: Approved Standard -M02-A11, Vol 32, and No1. Eleventh edition.