

Isolation and Antimicrobial sensitivity pattern of *Klebsiella pneumoniae* from sputum samples in a tertiary care hospital

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Abstract

Background: 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 causes 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. This study was done to determine the isolation rate of *Klebsiella*, their antibiogram and for the presence of resistant strains from sputum samples.

Materials & Methods: A total number of 128 sputum samples were included in the present study. A total of 128 organisms were isolated, of them 30 (23%) were *Klebsiella pneumoniae*. *Klebsiella* were identified by standard microbiological techniques and antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method and interpreted as per CLSI guidelines. A total of 30 *Klebsiella* spp were isolated from 128 sputum samples, 100% were *Klebsiella pneumoniae*, sensitive to Amikacin (66%) Ciprofloxacin (68%), Gentamicin (62%), Cefepime (60%), Imipenem (56.66%), Aztreonam (52.63%). Isolates showed high resistance to Ticarcillin clavulanic acid (81%), Tobramycin (58%), Co-trimoxazole (50%).

Results: The present study reveals the frequency of isolation of *Klebsiella* from sputum samples and their tendency towards antibiotic resistance. *K. pneumoniae* was found to be most sensitive to Amikacin, Gentamicin, Cefepime, Imipenem, Aztreonam and Ciprofloxacin. Females above 60 years of age are more affected and infections are more commonly associated with chronic obstructive pulmonary diseases and pneumonia.

Conclusion: 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.

Keywords: *Klebsiella pneumoniae*, Antibiotic resistance, Antibiotic susceptibility testing, Sputum.

1. Introduction

Currently, drug resistance to human pathogenic bacteria is frequently being reported worldwide. However, the situation is alarming in both developing as well as developed countries due to injudicious use of antibiotics inspite of pharmaceutical industries producing large number of newer antibiotics in the last three decades [1]. Microorganisms develop resistant to both older and newer antibiotics. Bacteria are known to have an increase ability to transmit and acquire resistance to these therapeutic drugs and also transferring the resistance from one bacteria to other resistant bacteria poses a threat to public health in such a way thereby increasing morbidity and mortality [2].

Antibiotics were considered to be the most effective therapeutic agents to combat microbial infections. But as a result of the overuse and injudicious use of antibiotics, these have been an emergence and spread of

multidrug resistant strains among different groups of microorganisms. Infections resistant bacteria are emerging threat all over the world both as hospital acquired as well as community acquired microorganisms [3]. In 1883 Friedlander isolated a capsulated bacillus from the lungs of patient who had died of pneumonia. The microorganism was named after him as Friedlander s bacillus. Later it was recommended as *Klebsiella*, and is reported worldwide.

Klebsiella is a Gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the *Enterobacteriaceae* family. 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. In both community and hospital settings resistant bacteria are emerging as a worldwide threat even in common infections. It is known to produce community acquired pneumonia

especially in chronic alcoholics, urinary tract infection, wound infections, blood infections and infections in the intensive care unit [4].

Klebsiella is known to have several virulence factors such as capsular polysaccharides, lipo polysaccharide (LPS) and iron-scavenging systems (siderophores). Nosocomial isolates are frequently resistant to numerous antibiotics as a result of the acquisition of multidrug resistance (MDR) plasmids.

It is essential to understand the antimicrobial susceptibility pattern of *Klebsiella* which shows variation in different geographical settings, in order to implement effective control measures to prevent rapid spread of drug resistance [5]. The present study was done as there was an increased prevalence of *Klebsiella Pneumoniae* in sputum from this tertiary rural hospital and also to carry out a rational selection of antimicrobials in our hospital.

2. Materials and Methods

This study was conducted at Akash Institute of Medical Sciences & Research Centre, Devanahalli, Bangalore rural. Total of 128 sputum samples were collected and subjected to antibiotic sensitivity in Department of Microbiology from inpatient and outpatient departments of our rural tertiary Akash Hospital from period Jan 2015 to June 2015. Sputum samples were collected in a sterile leak proof container and were processed according to standard conventional methods and the isolates were identified by standard biochemical tests.

2.1 Characterization of bacterial isolates

Sputum samples were aseptically inoculated on to Blood, Chocolate and Mac Conkey agar plates and incubated overnight at 37°C. *Klebsiella pneumoniae* (*K.pneumoniae*) isolates were identified by their morphology and biochemical characteristics. Morphology of *Klebsiella* identified were large dome shaped colonies on Blood, Chocolate agar and lactose fermenting mucoid colonies on Mac Conkey agar. In Gram staining, Gram negative, short, stout, blunt rods were seen. The biochemical characters identified were negative Indole test, 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 [6].

2.2 Antimicrobial susceptibility testing

A total of 30 positive isolates were screened for antimicrobial susceptibility testing by Kirby-Bauer disc

diffusion method on Mueller-Hinton agar (Hi-Media) and interpreted as per CLSI guidelines [7]. A log phase broth culture inoculum of the isolate with a turbidity equivalent to McFarland 0.5 standard (1.5×10^8 CFU/ml) was prepared and lawn cultured on the Mueller-Hinton agar and allowed to dry. Antibiotic discs were applied to the Mueller Hinton agar surface with the help of sterile forceps. The antibiotics chosen for the study were Imipenem (10µg), Gentamicin (10µg), Amikacin (30µg), Cotrimoxazole (25µg), Aztreonam (30µg), Tobramycin (10µg), Ciprofloxacin (30µg), Ticarcillin/Clavulanic acid (30µg) (Hi-Media). A panel of antibiotics which was selected as per the CLSI guidelines, but only the antibiotics which were showing higher degree of sensitivity were included in this study. All antibiotics used were on ATCC strains, to ensure that the discs were working.

The plates were then incubated at 37°C for 24 hours. Antimicrobial activity was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter using a calibrated scale. An organism was interpreted as 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, which were compared with the zones of control strains as demonstrated in the Kirby-Bauer chart [8]. Antimicrobial susceptibility of *K. pneumoniae* to different antibiotics is obtained. From antimicrobial susceptibility, antibiogram for *K. pneumoniae* is prepared and results are interpreted, considering the antimicrobial susceptibility probable drug of choice is selected for *K. pneumoniae*.

3. Results

During the 6 month period, a total of 128 sputum samples were processed for culture and sensitivity testing. Sputum samples of patients of all age groups (1day to >60years) both sexes were processed. A total 30 *Klebsiella pneumoniae* were isolated thus culture positivity was 23.43%, of the 30 *Klebsiella pneumoniae* isolates, 11 were from males and 19 were from females. Isolation rate was highest in patient aged above 60 years (male 31.57% & female 45.45%) followed by 45-60 years of males (36.36%) and females (21.05%). Lowest rate of isolation was observed in male (9.09%) and female (15.78%) aged 16-30 years. Table 1 shows the distribution of *Klebsiella pneumoniae* infection in various age groups [9].

Table 1: Frequency of age and sex wise distribution

Age in years	Males (N=58)	Female (N=70)	No. of Culture positives Males (N=11)	No. of culture positives Females (N=19)
0-15	10	9	0	0
16-30	10	15	1 (9.09%)	3 (15.78%)
31-45	9	10	1(9.09%)	6 (31.57%)
45-60	10	16	4(36.36%)	4 (21.05%)
>60	19	20	6 (31.57%)	5(45.45%)

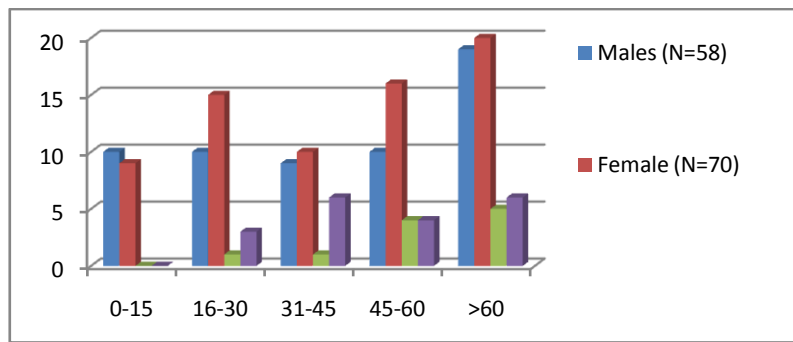


Fig I: Frequency of age and sex wise distribution of *Klebsiella pneumoniae* infection

Table2: Isolation of *Klebsiella pneumoniae* from sputum samples

Samples	Total no. of samples	No. of Culture positives
Sputum	128	30 (23.43%)

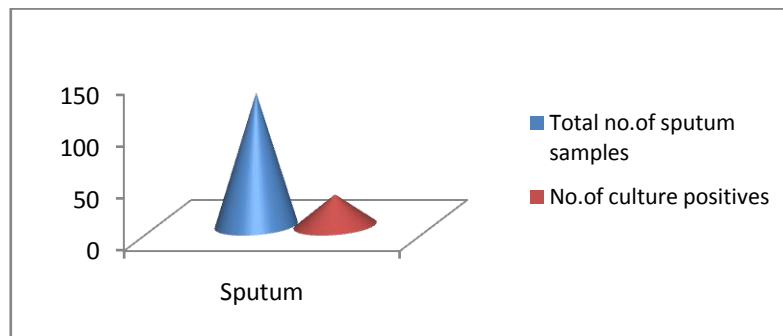


Fig II: Isolation of *Klebsiella pneumoniae* from sputum samples

Antimicrobial susceptibility testing revealed isolates were sensitive to Amikacin (66.66%), Ciprofloxacin (68.90%), Gentamicin (62.50%), Cefepime (60.00%), Imipenem (56.66%), Aztreonam (52.63%) (Fig. III). Isolates

were highly resistant to Ticarcillin/clavulanic acid (81.48%), Tobramycin (58.06%). Co-trimoxazole (50.0%). Antimicrobial susceptibility pattern of *K. pneumoniae* is shown in Table 3 [10].

Table 3: Antimicrobial sensitivity of *Klebsiella pneumoniae* isolated from sputum samples

Antibiotics sensitivity pattern	Resistant	Sensitive
Gentamicin	37.50%	62.50%
Co-trimoxazole	50.00%	50.00%
Amikacin	33.33%	66.66%
Ciprofloxacin	31.03%	68.90%
Cefepime	40.00%	60.00%
Imipenem	43.33%	56.66%
Ticarcillin/clavulanic acid	81.48%	18.51%
Tobramycin	58.06%	41.93%
Aztreonam	47.36%	52.63%

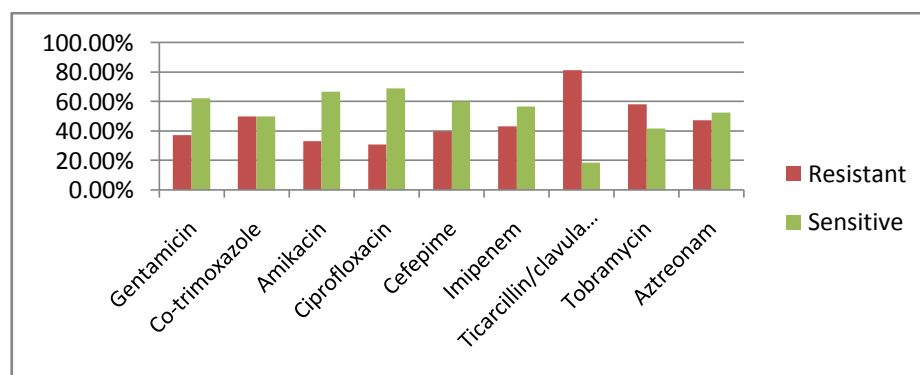


Fig. III: Antimicrobial susceptibility pattern of *Klebsiella pneumoniae*

4. Discussion

The present study reveals that, *Klebsiella pneumoniae* infection was predominant organism isolated from females (45.45%) than males (31.57%), *Klebsiella* infection was more commonly seen in persons aged 45-60 and above 60 years of age [11]. A female dominance of infection was observed in age group 16-30 years (15.78%), 31-45 years (31.57%) and >60 years (45.45%) than males (9.09%, 9.09% and 31.57% respectively). The higher infection in females of >60 years were mainly associated with chronic obstructive pulmonary diseases (COPD), pneumonia and other respiratory diseases [12].

Present study highlights the most alarming situation of highly diverse antibiotics resistance. In our study, 91.66% of the isolates showed resistance to Ampicillin. The chromosomally encoded β -lactamases could be responsible for this intrinsic resistance [13]. Among the fluoroquinolones tested, Ciprofloxacin (68.90%) was the most effective antibiotic against *Klebsiella pneumoniae*. Aminoglycosides such as Amikacin (66.66%) and Gentamicin (62.50%), Cephalosporins cefepime (60.66), β -lactam antibiotics Imipenem were the most effective antibiotic agents against *Klebsiella pneumoniae* [14].

A significant high resistance to Ticarcillin/clavulanic acid (81.48%), Tobramycin (58.06%) there are reports covering high levels of resistance of *K. pneumoniae* towards these antibiotics in other countries also. 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 [15]. Alarming finding seen in this study was that resistance shown to Ticarcillin/clavulanic acid. Overall resistance 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 [16]. Another mechanism is associated with penicillin-binding protein 2a (PBP2a), encoded by *mecA2*. Another gene involved in penicillin resistance in staphylococci is *blaZ* which encodes β -lactamase [17]. In present study, *K. pneumoniae* showed least resistance to Amikacin, Ciprofloxacin and Gentamicin. 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 Gentamicin mean that there is a possibility of sensitivity to other aminoglycosides such as Streptomycin, neomycin and Kanamycin [18].

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 [19]. 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 [20].

5. Conclusion

In conclusion, 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 and mortality rates. *K. pneumoniae* infection was predominant in females, 31-45 years and above 60 years of age. The common predisposing factors associated with *Klebsiella pneumoniae* infection were chronic obstructive pulmonary diseases (COPD), pneumonia and other respiratory diseases. *K. pneumoniae* was found to be most sensitive to Amikacin, Gentamicin, Ciprofloxacin, Cefepime, Imipenem and Aztreonam. Considering the antibiogram, Amikacin, Gentamicin, Cefepime, Imipenem and Ciprofloxacin should be preferred drugs for *K. pneumoniae* infection isolated from sputum. Regular surveillance of antibiotic susceptibility pattern may help to overcome the indiscriminate use of antibiotics a major cause of emergence of drug resistance among pathogens and to develop antibiotic policies. 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.

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