Prevalence of Antimicrobial Drug Resistance of *Klebsiella pneumoniae* in India

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Abstract—*Klebsiella pneumoniae* has been associated with different types of infections and one of the most important aspects of *Klebsiella* is the emergence of multi-drug resistant strains particularly those involved in nosocomial diseases. Fifty – nine clinical isolates were collected from different parts of India. Most of the samples were recovered from respiratory, urinary tract infection and pus cases which were followed by biochemical characterization. Twenty confirmed *K. pneumoniae* isolates were further tested for antimicrobial drug sensitivity and almost fifty percent of them were found to be multidrug resistant. As per our statistical data, all confirmed *K.pneumoniae* isolates were resistant to carbenicillin and one among them recovered from sputum sample of a pneumonic patient was resistant to all the antimicrobial agents tested except exhibiting a partial susceptibility to amikacin. In our studies we found that *K.pneumoniae* strains from clinical cases were highly susceptible to quinolones and the aminoglycoside, amikacin and gentamycin. At the same time over 60 % strains were resistant to chloramphenicol and tetracycline. We also found that 28 to 76 % of them were resistant to cephalosporins (ceftizoxime and cefotaxime). On the basis of statistical binomial test we conclude that piperacillin, carbenicillin, ofloxacin ampicillin, co-trimoxazole and chloramphenicol were significantly resistant, whereas cefotaxime and tetracycline were found to be moderately resistant against *Klebsiella pneumoniae*.

Index Terms—Antibiotic, antimicrobial drug resistance, *Klebsiella pneumoniae*

I. INTRODUCTION

In 1883 Friedlander isolated a capsulated bacillus from the lungs of patient who died of pneumonia. 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. Strains of *Klebsiella* are responsible for a wide variety of diseases in humans. These bacteria have become important pathogens in nosocomial infections [1] which have been well documented in United States [2] and India [3]. Epidemic and endemic nosocomial infections caused by *Klebsiella* species are leading causes of morbidity and mortality [4]. In addition to being the primary cause of respiratory tract infections, it is also commonly involved in acute pyelonephritis in pregnant women with urinary tract abnormalities such as urolithiases, hydronephrosis or congenital deformities. They may induce sepsis and have a marked tendency to exist as mixed infections or as secondary infections with other pathogenic bacteria [5]. Recently, World Health Organization also warned the community that multidrug resistant bacteria are emerging worldwide which is a big challenge to healthcare. If we didn’t take immediate action then antibiotics may lose their power to cure diseases [6]. 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 both increased carriage of *Klebsiella* and the development of multidrug-resistant strains that produce extended–spectrum beta-lactamase (ESBL). Antimicrobial chemotherapy depends on the concept that pathogens differ from their hosts in some feature of their biochemistry that renders them susceptible to attack with chemicals that have no, or few, deleterious effects on the host. In effect, chemotherapy requires selective toxicity[7]. Antibiotics are chemically diverse, although there are major families such as the β-lactams, aminoglycosides, polyketides, and macrolides.

Epidemic strains of cephalosporin resistant *K.pneumoniae* have been associated with increased morbidity and mortality in hospitalized patients [8]. Since 1983, nosocomial outbreaks of ESBL producing *K.pneumoniae* infections in Europe [9], the United States and South America have been described [10]. Between 1990 and 1992, 5% of *K.pneumoniae* clinical isolates produced ESBLs [11]. In France, 10 to 30 % of *K.pneumoniae* strains are reported to produce plasmid mediated ESBLs of the TEM or SHV families [12]. Generally, multidrug resistant bacteria are categorized into the Gram positive, Gram negative and acid fast bacilli [13, 14 and 15]. Emergence of Multi-drug resistant bacteria is associated with four resistant strategies used by bacteria that diminish the effects of antibiotics. First one is based on enzymatic modification and inactivation of antibiotics, second is restriction of drug targets access, third is alteration of drug target or even complete diminish of the target and last one is based on phenotypic resistance [16]. We have started our studies with three main objectives. First one is to analyze the severity of multidrug resistance of *Klebsiella pneumoniae* in India. Second one is to know the challenges faced by healthcare personnel during treatment of multi drug resistant *K.pneumoniae* patients and the last one is to come up with some suggestions to overcome multidrug resistant problem.
II. MATERIAL AND METHODS

We have collected standard and clinical isolates of \textit{K. pneumoniae} from different places of India and utilized them for their antibiotic resistant ability.

\textbf{A. Bacterial strains}

Standard culture of \textit{K. pneumoniae} strain 3296 was collected from Yamaguchi University, Japan whereas total fifty nine clinical isolates were collected from different places of India. Out of all, fifty - four clinical isolates of \textit{Klebsiella} species were recovered from pus, urine and sputum samples from Armed Force Medical College, Pune, India, four samples from pneumonic patients and one urinary tract infection sample from Patel Chest Hospital, New Delhi, India.

\textbf{B. Biochemical characterization}

All clinical isolates were examined morphologically for colony characteristics on agar media. Those exhibiting mucoid colonies were processed for biochemical testing. Biochemical test employed were urease production, citrate utilization and fermentation of sugars. Sugar fermentation tests performed were sucrose, glucose, mannitol, lactose, adonitol, dulcitol, melibiose and esculin. Indole test and \textit{H}_2\textit{S} production on TSI agar, oxidase, catalase and nitrate were also carried out. Besides these tests, motility and growth of organism in potassium cyanide were also checked. For biochemical tests standard procedures were used [17].

\textbf{C. Antibiotic sensitivity}

Antibiotic sensitivity of clinical \textit{K. pneumoniae} isolates was done by Bauer’s and Kirby’s disc diffusion method [18]. Organisms were grown in BHI broth and inoculated on Mueller Hinton agar plates by sterile swabs and then antibiotic discs were placed on media and pressed gently followed by overnight incubation. The antibiotics that were tested included Ampicillin (20mcg), Cotrimoxazole (25mcg), Piperacillin (100mcg), Gentamycin (10mcg), Amikacin (30 mcg), Carbenicillin (100mcg), Cefotaxime (30mcg), Ceftriaxone (30mcg), Tetracycline (30mcg) and Ofloxacin (5 mcg).

III. RESULTS

The standard and clinical isolates of \textit{Klebsiella} were examined by battery of biochemical tests. Standard \textit{K. pneumoniae} cultures showed positive reactions for urease production, citrate utilization, catalase reaction and fermentation of sugars like glucose, lactose, sucrose, mannitol, adonitol, melibiose and esculin. Organisms showed negative test for indole production. There was no \textit{H}_2\textit{S} production on Triple Sugar Iron agar but growth of organism was seen in potassium cyanide. All fifty-nine clinical isolates exhibiting colonies similar to \textit{Klebsiella} species were tested biochemically. Thirty-six clinical isolates showed typical common biochemical reaction pattern similar to the one seen with \textit{Klebsiella} species, being positive to glucose, lactose, sucrose, mannitol and negative to oxidase and indole. When tested by second battery of biochemical reactions that included some rare sugars (adonitol, melibiose, esculin and dulcitol), twenty of them exhibited reactions attributable to majority of \textit{K. pneumoniae} sub species pneumoniae strain, being positive to adonitol, melibiose, esculin, urease and citrate. They also showed growth in potassium cyanide. The rest of the isolates had variable reactions with these tests. Results of biochemical tests are shown in table 1.

Antibiotic sensitivity testing of twenty confirmed \textit{K. pneumoniae} clinical isolates was done on Muller – Hinton agar plates. On the basis of resistance to antibiotic, strains were categorized into three groups i.e. susceptible(S), resistant (R) and moderately susceptible (MS) as shown in table 2. We have used thirteen antibiotics which includes Ampicillin (AS), Co-trimoxazole (BA), Cefotaxime (CF), Piperacillin (PC), Chloramphenicol (CH), Ciprofloxacin (CP), Ceftriaxone (CL),Tetracycin (TE), Ofloxazina (OF), Gentamicine (GM), Amikacin (AK), Pefloxan (PF) and Carbenicillin (CN). All of these antibiotics were categorized into three categories on the basis of their sensitivity. Results of one group had strains which were susceptible (over 85%) to quinolones and aminoglycosides. The second group had strains which were moderately susceptible (intermediate) to antibiosomal antibiotics (chloramphenicol and tetracycline) with 62% resistant strains. The third group contained strains which were resistant to semi- synthetic penicillins, ampicillin, carbenicillin (76-100%) and to co-trimoxazole (76%). Almost five antibiotics ofloxacin, gentamycine, amikacin, pefloxan and ciprofloxacin. showed susceptibility to \textit{Klebsiella pneumoniae} whereas seven antibiotics carbenicillin, piperacillin, ampicillin, Co-trimoxazole, cefotaxim, chloramphenical and tetracycline showed significant resistance against \textit{Klebsiella pneumoniae}. Results are shown in fig 1.

IV. DISCUSSION

In vitro data showed a wide range of beta-lactams, aminoglycosides, quinolones and other antibiotics which are useful for treatment of \textit{Klebsiella} infections [19,20 and 21]. Both Gram positive and Gram negative bacteria have cell walls which is composed of heavily cross-linked peptidoglycan layers which are catalysed by cell-wall
transpeptidases also known as penicillin binding protein (PBP). B-lactam antibiotics disturb peptide bond formation by acting as competitive inhibitors to these PBPs. These result in formation of irreversible covalent bonded penicilloyl-enzyme complexes with weak cross-linked peptidoglycans, thus ease bacteria lyses and death [22]. All confirmed clinical isolates of \( \textit{K}.\textit{pneumoniae} \) were tested for antimicrobial sensitivity. Fifty four percent of them were found to be multidrug resistant. All the \( \textit{Klebsiella pneumoniae} \) isolates were resistant to carbenicillin and one among them recovered from sputum sample of a pneumonic patient was resistant to all the antimicrobial agents tested except exhibiting a partial susceptibility to amikacin. In such cases the disease is prone to progress to permanent debilitation or death of the patient if, isolation and identification of the causative agent and the subsequent antimicrobial susceptibility testing is not carried out at the early stage of the disease.

In our studies, \( \textit{K}.\textit{pneumoniae} \) strains from clinical cases were found highly susceptible to quinolones and aminoglycoside, amikacin and gentamycin. At the same time over 60% strains were found resistant to chloramphenicol and tetracycline. Twenty-eight to 76% of them were resistant to cephalosporins (ceftizoxime and cefotaxime). Cephalosporins have been widely used as monotherapy and in combination with aminoglycosides for the treatment of Klebsiella infection. Plasmid encoded resistance to broad spectrum cephalosporins is becoming a widespread phenomenon in clinical medicine. These antibiotics are inactivated by an array of different extended spectrum beta lactamases (ESBLs) which have evolved by stepwise mutation of TEM/SHV type beta lactamases. Plasmid encoding these enzymes has been encountered in several members of the family enterobacteriaceae, but are, for unknown reasons, most often harboured by \( \textit{K}.\textit{pneumoniae} \). Epidemic and endemic nosocomial infections caused by ESBL producing \( \textit{K}.\textit{pneumoniae} \) represent a persistent problem in many parts of the world, especially in ICUs [23,24].

The emergence of multidrug resistant strains particularly those involved in nosocomial diseases and the alarming rise in resistance to SHV and ESBL producing groups of antibiotics result in high morbidity and mortality. Early identification of agent, therefore, is important for timely management of patients.Klebsiella has been associated with different types of infections and one of the important aspects of Klebsiella associated infection is the emergence of multi-drug resistant strains particularly those involved in nosocomial diseases. The alarming rise in resistance to SHV and ESBL producing groups of antibiotics result in high morbidity and mortality. TEM- and SHV type ESBL producing \( \textit{K}.\textit{pneumoniae} \) were extensively reported worldwide after it was first identified in enterobacterial isolates from India. The high prevalence of these drug resistant strains has further necessitated the requirement of a rapid and accurate identification system for \( \textit{K}.\textit{pneumoniae} \). We have found that the isolates were highly susceptible to quinolones and the aminoglycosides. Over sixty percent strains were resistant to chloramphenicol and tetracycline but all the isolates were resistant to carbenicillin.
V. CONCLUSIONS

Statistical data and evidences from researches prove that multi drug resistant bacteria are emerging worldwide which causes many public health problems and challenges to healthcare. Antimicrobial resistance is a global concern not only because it kills but because it increases health costs and threatens patient care [6]. Moreover, uses of broad spectrum antibiotics, insufficient aseptic condition and technique with inadequate control of infections spread had aggravated this problem. To conclude our research findings data analysis was done using SPSS Package version 18 and the p value of less than 0.1 was considered statistically significant. On the basis of binomial test, we conclude that piperacillin (P=0.000), carbencillin (P=0.000), oxoflaxine (P=0.000), ampicillin (P=0.096), co-trimoxazole (P=0.96), and chloramphenicol (P=0.035) were significantly resistant, whereas cefotaxime and tetracycline were found to be moderately resistant against Klebsiella pneumoniae. To overcome multidrug resistant problem, we would like to suggest that future research plans should focus on the genetic makeup of all multidrug resistant bacteria to understand more about their genes mutations and the effects on antibiotics resistance. Researches on rapid detection of infectious microorganisms should be encouraged. We should also take immediate action to strengthen surveillance and laboratory capacity. Physicians should also promote rational use of medicines to avoid antibiotic drug resistance. All healthcare professionals should work together with pharmacist and laboratory personnel to overcome this problem.

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REFERENCES

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