ANTIMICROBIAL SENSITIVITY PATTERN OF KLEBSIELLA PNEUMONIAE ISOLATED FROM SPUTUM FROM A TERTIARY HOSPITAL, CALICUT, KERALA.

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ABSTRACT: Antibiotic resistance is a serious hazard to public health that demands urgent preventive measures. It threatens the effective management of ever increasing infections caused by pathogens. This study is done to find out the antibiotic sensitivity pattern of Klebsiella pneumoniae (K. Pneumoniae) isolated from sputum and to highlight the evolving antimicrobial resistance. A total of 500 sputum samples were collected from patients who presented with respiratory tract infections (RTI) and examined. Nearly 30% of the samples were culture positive for various organisms. The most common pathogen isolated was K. Pneumoniae. Other organisms were Streptococci, Staphylococcus aureus and Pseudomonas species. K. Pneumoniae was found to be most sensitive to Imipenem, Amikacin and Gentamicin. Considering the antimicrobial susceptibility, cost and side effect profile, Ciprofloxacin and Cotrimoxazole are preferred drugs for outpatient treatment, while aminoglycosides are preferred for inpatient treatment of K. Pneumoniae infections.

KEYWORDS: K. Pneumoniae, Antimicrobial susceptibility, Antimicrobial resistance.

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INTRODUCTION: Antibiotic resistance has become a serious public health issue worldwide. The unscientific use of antibiotics is one of the main reasons for this phenomenon. Since there is a significant drop in the discovery of newer antimicrobial agents, the infections caused by resistant strains lead to increased mortality and morbidity than that caused by nonresistant strains.[1,2] This can lead to prolonged hospital stays, increased healthcare costs and increased mortality.[3]

K. Pneumoniae is a gram negative, non-motile, encapsulated, facultative anaerobic, rod-shaped bacterium. It is the most significant member of the Klebsiella genus of Enterobacteriaceae. Although it is found in the normal flora of mouth, intestine and skin,[4] it can cause serious respiratory tract, urinary tract and wound infections. It expresses two types of antigens on its cell surface. The first, O antigen is a component of the lipopolysacharide and second, K antigen, a capsular polysaccharide.[4] Klebsiella infections are seen most commonly in people with impaired defenses, like those with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary disease, glucocorticoid therapy and renal failure.

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Antimicrobial resistance of Klebsiella has become a major threat to the health care system. Current evidences depict plasmids as the major source of resistant genes.[4] With the ability to produce Extended Spectrum Beta Lactamase (ESBL), Klebsiella has become resistant to multiple antibiotics like aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and trimethoprim/ sulfamethoxazole.[5] Nowadays, infection with carbapenem-resistant Enterobacteri-aceae has become a challenge,[6] especially carbapenem-resistant Klebsiella pneumoniae (CRKP) or Klebsiella Pneumoniae Carbapenemase (KPC) producers. A number of mechanisms cause carbapenem resistance in Enterobacteriaceae. The most important mechanism in CRKP is the production of carbapenemase enzyme, KPC, by a gene called blaKPC.[7]

AIM OF THE STUDY: The aim of this study is to find out the antibiotic susceptibility pattern of K. pneumoniae isolated from sputum in a tertiary Centre- KMCT Medical College, Mukkom, Calicut, Kerala State (KMCTMC)-and to suggest suitable antibiotics to treat such infections.

METHODS: In this study sputum samples were collected from 500 patients with RTI and examined in the Department of Microbiology, KMCTMC from January 2013 to August 2015. To culture the Klebsiella, Half plate streaking method is done using chocolate blood agar and Mac Conkey agar and incubated overnight at 37^0C. K. Pneumoniae strains were identified by their morphology and biochemical characteristics. They were large, mucoid, dome-shaped colonies on blood agar and lactose

fermenting colonies on Mac Conkey agar. They were gram negative, straight rods. The biochemical characteristics include negative indole test, positive urease test and abundant gas production from glucose, lactose and sucrose fermentation tests.

Antibiotic susceptibility test was done for all the isolates using Mueller-Hinton agar by modified Kirby-Bauer disc diffusion technique. The antibiotic discs used were Ampicillin, Cefipime, Ceftriaxone, Cefazoline, Ciprofloxacin, Cotrimoxazole, Amoxicillin/Clavulanic acid, Cefuroxime, Gentamicin, Amikacin and Imipenem.

In this technique, firstly, the K. pneumoniae were inoculated in to peptone water and incubated at 37^0C. The turbidity was adjusted to 0.5Mc Farland standards which were used for antibiotic sensitivity testing. Using a sterile swab the cultures from liquid media were inoculated on Mueller-Hinton agar by lawn technique. A maximum of six disks were applied on the lawn and allowed at room temperature for 15 minutes for diffusion of antibiotics. Then the Mueller-Hinton agar plates were incubated at 37^0C for 16 to 18 hours. The results were read after the incubation time. The antibiotic sensitivity was indicated by zone of inhibition around each disk. The zone diameters were measured in millimeter (including disk diameter) and categorized in to susceptible, intermediate susceptible and resistant categories as per CLSI (Clinical and Laboratory Standard Institute) guidelines.[8]

RESULTS: The given Table 1 and bar diagram depict that K. pneumoniae, isolated from sputum, is most sensitive to Imipenem, Amikacin, Gentamicin, Ciprofloxacin and Cotrimoxazole respectively. K. pneumoniae is least sensitive to Amoxicillin/Clavulanic acid, Ampicillin, Cefazoline, Cefipime, Ceftriaxone, Cefotaxim and Cefuroxime respectively. K. pneumoniae is less than 50% sensitive to all antibiotics other than Imipenem and Amikacin.

DISCUSSION: This study was performed to identify the susceptibility of K. pneumoniae, isolated from sputum samples of patients who presented with RTI, towards 12 antibiotics, in a tertiary centre, KMCTMC, and to report the evolving antibiotic resistance by this bacteria in this part of country. The proportion of antimicrobial susceptibility towards these antimicrobial agents is shown in Table 1 and Figure 1.

It is disturbing to notice that Klebsiella is sensitive only to antibiotics such as Imipenem, Amikacin and Gentamicin and most of them are multi drug resistant. Out of these drugs only the first two boasted an impressive sensitivity of 81.58 and 59.2% respectively. K. pneumoniae is least sensitive to Amoxicillin/Clavulanic acid and Ampicillin, showing a resistance of 97.37 and 96.05% respectively. These findings highlight that these multidrug resistant microbes can cause increased mortality, hospital stay and health care cost, unless they are detected and treated earlier with appropriate antibiotics.

This study also shows that Klebsiella is 40.8% sensitive to Cotrimoxazole, which is a much higher value when compared to the study conducted by Asati Rakesh Kumar et al.[9] This may be due to decreased use of older antimicrobials like Cotrimoxazole and over use of newer antibiotics resulting in a dramatic fall in the resistance towards older agents.

Moreover Klebsiella expresses increased sensitivity towards Imipenem, Amikacin and Gentamicin. Thus it can be assumed that they may also be sensitive to other carbapenems and aminoglycosides. The resistance of Klebsiella towards Imipenem is 18.42%, which is slightly lower than the figure in the study conducted by KN Ravichithra et al,[10] However it is still a very high value in this part of country where carbapenems are not used routinely. Infection with carbapenem-resistant Enterobacteriaceae (CRE) or carbapenemase-producing Enterobacteriaceae is emerging as an important challenge in health-care settings.[11] So we need to be very cautious about CRKPs, which can cause a very high mortality and morbidity. Considering both the cost and benefits, aminoglycosides are thought to be better drugs for K. pneumoniae infections.

Infections with CRKPs were associated with organ/stem cell transplantation, mechanical ventilation, exposure to antimicrobials, and overall longer length of stay in hospitals.[12] In February 2015, the FDA reported about a transmission risk when people undergo a gastroenterology procedure called endoscopic retrograde cholangio pancreatogram (ERCP. If incompletely disinfected, the device can transmit CRE from one patient to another[13] According to one study, exposure to antibiotics, especially Fluoroquinolones, and previous hospitalization dramatically increased the risk of acquisition of Ertapenem (a Carbapenem antibiotic) resistant bacteria.[14]

Although individual cephalosporins differ in their antibacterial spectrum, relative potency against specific organisms, susceptibility to beta lactamase and pharmacokinetic properties, Klebsiella shows high resistance to these agents especially to third generation cephalosporins. This is mainly due to the production of extended spectrum beta lactamase (ESBL) and metallic beta lactamase (MBL), which can be encoded chromosomally or plasmid mediated, by K. pneumoniae. Addition of betalactamase inhibitors along with beta lactams can reduce the dose, toxicity and resistance towards the latter.[15,16]

K. pneumoniae can cause severe damage to the human lungs through inflammation, hemorrhage and cell death which can cause thick, bloody, mucoid sputum called currant jelly sputum. The most common condition caused by Klebsiella outside the hospital is pneumonia, which can be easily complicated to lung abscess, cavitation, empyema and pleural adhesions. The spectrum of diseases includes pneumonia, urinary tract infections, thrombophlebitis, cholecystitis, diarrhea, upper respiratory tract infections, wound infections, osteomyelitis, meningitis, bacteremia and septicemia.

Klebsiella has become a multidrug resistant organism due to its plasmids which act as the primary source of resistance genes in these microbes, especially those with the ability to produce extended-spectrum beta-lactamases (ESBL). They can develop and transfer beta lactam resistance through several ways such as generation of new ESBL through amino acid substitution of existing plasmid mediated beta lactamase, acquiring ESBL from environmental bacteria, increasing the expression of chromosome encoded beta-lactamase gene (bla gene) via regulator gene and promoter sequence modifications and mobilization of bla genes through integrons or horizontal transfer of genomic islands to other strains.[17]

CONCLUSION: In this study an effort is made to detect antimicrobial susceptibility of K. pneumoniae isolated from sputum of patients who presented with RTI and to evaluate the antimicrobial resistance. It is found that Klebsiella is sensitive only to very few antibiotics such as Imipenem, Amikacin, Gentamicin, Ciprofloxacin and Cotrimoxazole. Even though they are generally sensitive to Carbapenems, they have started showing significant resistance towards this group of antibiotics, raising the probability of existence of CRKPs in this area. Since Carbapenems are considered among the last choice antibiotics, we have to be very cautious about CRKPs.

There is also a significant increase in sensitivity towards older antibiotics like Cotrimoxazole, which shows that careful use of antimicrobials can hinder the progress of resistance.

If an organism is found to be sensitive to various antibiotics, we need to consider its cost, route of administration, side effects and patient's health status before starting it. Cheaper drugs with less side effects and oral route of administration should be preferred over others. Thus it can be concluded from our study that Ciprofloxacin and Cotrimoxazole are preferred drugs for outpatient treatment, while aminoglycosides are preferred for inpatient treatment of K. pneumoniae infections.

Hospitals are primary transmission sites for CRE-based infections. To prevent spreading Klebsiella infections between patients, healthcare personnel must follow specific infection-control practices. These precautions include strict adherence to hand hygiene and wearing gowns and gloves when they enter rooms where patients with Klebsiella–related illnesses are treated. Hospitals also must follow strict cleaning procedures to prevent the spread of Klebsiella.

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Antibiotic	Sensitivity (%)	Resistance (%)
Amikacin	59.2	40.8
Ampicillin	3.95	96.05
Cefipime	17.1	82.9
Ceftriaxone	25	75
Ciprofloxacin	44.73	55.27
Cefazolin	14.5	85.5
Cotrimoxazole	4o.8	59.2
Amoxiclav	2.63	97.37
Cefuroxime	25	75
Gentamicin	48.68	51.32
Cefotaxim	25	75
Imipenem	81.58	18.42

Table 1: Antibiotic Susceptibility of K. Pneumonia

No.	Antibiotic	Sensitivity (%)	Resistance (%)	Total cost of treatment for 7 days in rupees	Toxicity
1	Imipenem	81.58	18.42	8400	moderate
2	Amikacin	59.2	40.8	955.5	moderate
3	Gentamicin	48.68	51.32	68.18	moderate
4	Ciprofloxacin	44.73	55.27	58.8	moderate
5	Cotrimoxazole	4O.8	59.2	20.3	Moderate

Table 2

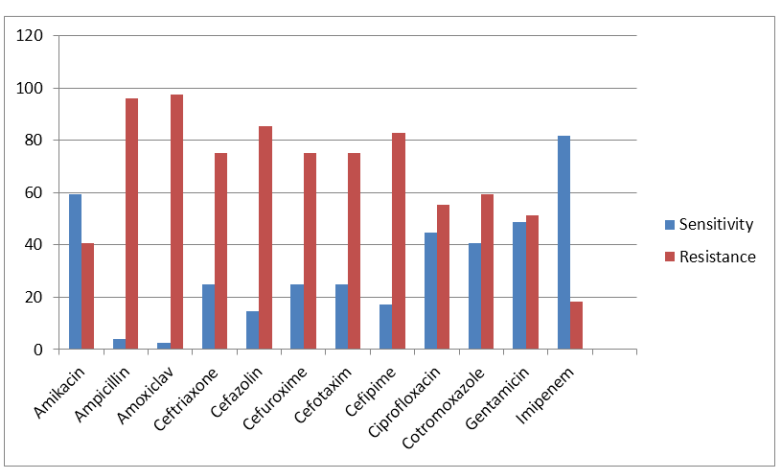

Figure 1. Antibiotic Susceptibility of K.pneumoniae