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Research Article

Antimicrobial Sensitivity Pattern of *Klebsiella Pneumoniae* isolated from Sputum from Tertiary Care Hospital, Surendranagar, Gujarat and Issues Related to the Rational Selection of Antimicrobials

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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. Respiratory tract infection (RTI) is common infection worldwide and numbers of patients are presenting to general practice and inpatient department. Both Gram positive and Gram negative bacteria are involved in causing RTI. *Klebsiella pneumoniae (K. pneumoniae)* is one of the most common causative agents of RTI and it has also become important pathogens in nosocomial infections causing RTI. This study is done to find out the prevalence and antimicrobial susceptibility pattern of *K. pneumoniae* isolated from sputum, causing respiratory tract infection in tertiary care hospital, Surendranagar, Gujarat. Total 512 sputum samples were collected and tested bacteriologically using standard procedures. Culture positivity of urine samples was found to be 29 %. The most common pathogens were *K. pneumoniae* (39.5 %) followed by *Pseudomonas* (25 %), *E. coli* (11.5 %), *Staphylococci* (11.5%) and others (3.8%). Antimicrobial susceptibility testing was done by disk diffusion method described by Kirby-Bauer (1961). *K. pneumoniae* is most sensitive for amikacin followed by gatifloxacin, chloramphenicol, cefipime, ciprofloxacin and cefoperazone plus sulbactam, if isolated from sputum. Considering the antibiotic susceptibility testing, cost, side effects and many other factors, gatifloxacin should be preferred for *K. pneumoniae* infection for RTI.
Keywords: Respiratory tract infection, sputum, *Klebsiella pneumoniae*, antibiotic susceptibility testing, antimicrobial resistance.

INTRODUCTION

Antimicrobial resistance has become a serious public health problem worldwide. Infections caused by resistant bacteria are associated with increased morbidity and mortality than those caused by susceptible pathogens [1, 2].Infections caused by resistant bacteria led to prolonged hospital stays, increased health care costs and in many cases to untreatable infections [3].

Klebsiella pneumoniae (K. pneumoniae) are ubiquitously present and reported worldwide. In recent years, *K. pneumoniae*have become important pathogens in nosocomial infections [4]. The importance of *K. pneumoniae* species in the ever increasing number of gram negative aerobic bacillary nosocomial infections in the United States [5] and India [6] has been well documented. Epidemic and endemic nosocomial infections caused by *K. pneumoniae* species are leading causes of morbidity and mortality [7]. In addition to being the primary cause of respiratory tract infections like pneumonia, rhinoscleroma, ozaena, sinusitis and otitis, it also causes infections of the alimentary tract like enteritis, appendicitis and cholycystitis.

Evidences from researches prove that multidrug resistance bacteria are emerging worldwide which is a big challenge to healthcare. Multidrug resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate using available antibiotics. Moreover, extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *K. pneumoniae* and the development of multidrug-resistant strains that produce extended–spectrum beta-lactamase (ESBL). Epidemic strains of cephalosporin resistant *K.pneumoniae* have been associated with increased morbidity and mortality in hospitalized patients [8].

Antimicrobial agents are among the most commonly used and misused of all drugs. 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 [9].

The microbiology laboratory plays a central role in the decision to choose a particular antimicrobial lagent over others. First, identification and isolation of the causative organism should be taken place in 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 [10].

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

MATERIAL AND METHODS

In the present study, 512sputum samples from were processed in Department of Microbiology from inpatient & outpatient department of C.U. Shah Medical College & Hospital Surendranagar; from period January 2007 to December 2008.

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 H_2Sproduction 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. Forbiochemical tests standard procedures were used [11]

Antibiotic Sensitivity Testing-

Antibiotic sensitivity testing (AST) was done only for pathogenic bacteria. Antibiotic sensitivity was performed by Disc Diffusion Method of Bauer et al[12]. 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[13].

Antibiogram for *K. pneumoniae* was developed from antibiotic sensitivity testing then on the basis of antibiotic sensitivity, cost effectiveness and ADR profile, appropriate antibiotic for treatment of *S. aureus,* isolated from different urine samples was achieved.

RESULT

Table 1*Number and % of organisms, isolated from sputum*

Serial Number	Organism	Number of Organism	% Organism
1	***Klebsiella pneumoniae***	**82**	**39.5**
2	*Pseudomonas*	**52**	**25**
3	*Staphylococci*	24	11.5
4	*E. coli*	24	11.5
5	*Streptococci*	18	8.7
6	Others	08	3.8
	TOTAL	208	100

In the present study, 512 sputum samples from were processed in Department of Microbiology from inpatient & outpatient department of C.U. Shah Medical College & Hospital Surendranagar; from period January 2007 to December 2008. Out of all, 40.6 % clinical isolates were recovered sputum samples.

Table 2 - Antibiotic Sensitivity of *Klebsiella Pneumoniae*:-

Antibiotics	Sensitivity in %	Resistance in %	Antibiotics	Sensitivity in %	Resistance in %
Amikacin	92.7	7.3	Ampicillin/sulb.	44.1	55.9
Gatifloxacin	73.2	26.8	Gentamicin	41.3	58.7
Chloramphenicol	58.5	41.5	Oxytetracycyline	39.4	60.6
Cefipime	53.3	46.7	Piperacillin	37.5	62.5
Ciprofloxacin	51.2	48.8	Cefuroxime	33.6	66.4
Cefoperazone	48.9	51.1	Nalidixic acid	30.7	693
Ceftriaxone	47.2	52.8	Cefadroxyl	30.7	69.3
Cotrimoxazole	47.2	52.8	Ceftizoxime	26.8	73.2
Cefotaxime	47.2	52.8	Ticarcillin/clav.	14.9	85.1
Ofloxacin	47.2	52.8	Tetracycline	14.9	85.1
Norfloxacin	44.1	55.9	Amoxicillin/clav.	11.5	88.5
Ceftazidime	44.1	55.9	Polymixin-B	4.9	95.1

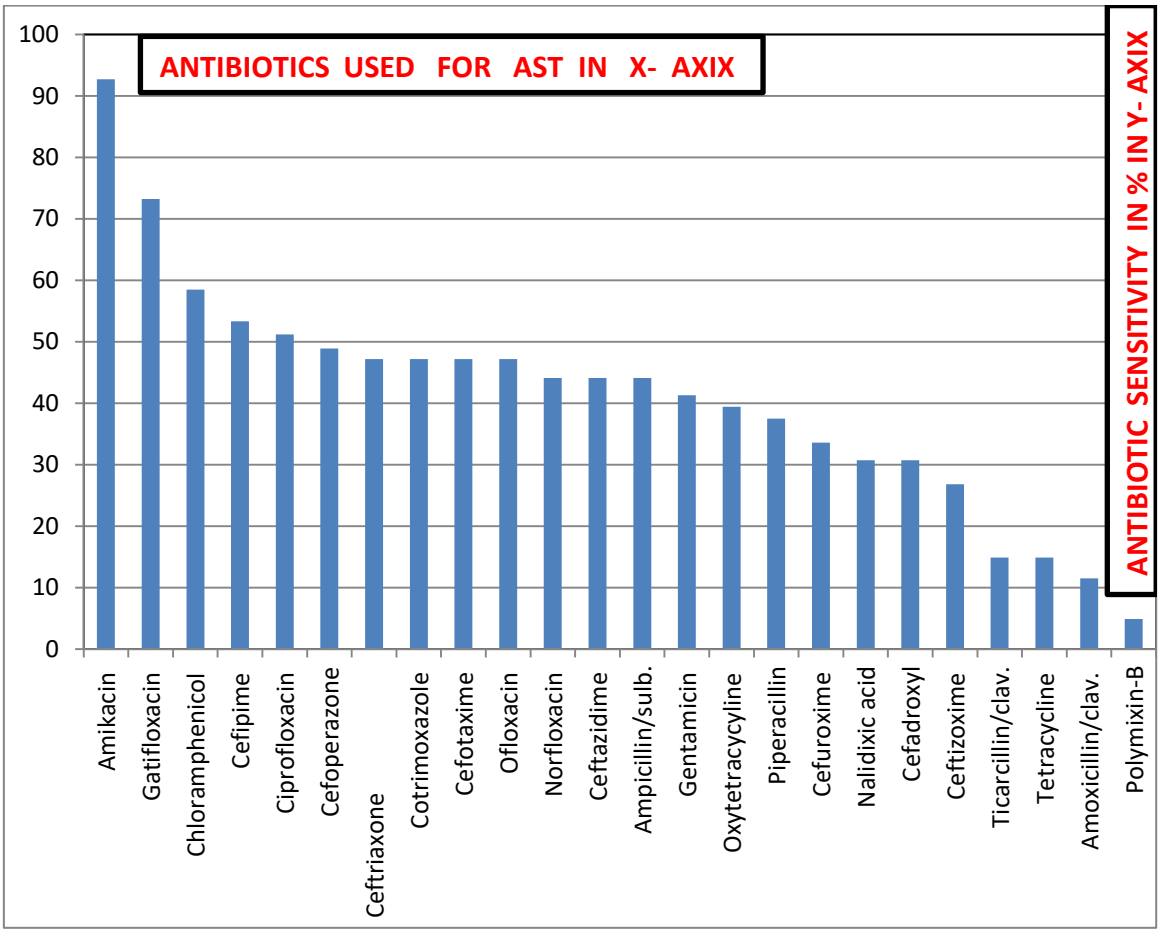

Figure 1 -Antibiotic Sensitivity of *Klebsiella Pneumoniae*:-

In above table-2 and figure 1, we observe that *Klebsiella pneumonia* (*K. pneumoniae)*is most sensitive for amikacin followed by gatifloxacin, chloramphenicol, cefipime, ciprofloxacin and cefoperazone plus sulbactam, if isolated from sputum. *K. pneumoniae* is less than 50% sensitive for other AMs. *K. pneumoniae* is least sensitive for ticarcillin/ clavulinic acid, amoxicillin/clavulinic acid and polymixin B.

Table-3 Drug of choice for *Klebsiella pneumoniae*isolated from sputum

Sr.no.	Name of drug	% Sensi.	Route of Adm.	Price in Rs Per 10 tab /per vial	Total duration of treatment	Total cost For treatment In Rs	ADR/ Toxicity of drug
1	**Amikacin**	92.7	IV/IM	10/500 mg vial	15mg/kg in 3 divided doses for 5 days	90-100	Mild – mod.
2	**Gatifloxacin**	73.2	Oral/ iv	50	500 mg OD ×7-10 days	35-50	mild
3	**Chloramphenicol**	58.5	Oral/iv	40	500 mg QID×7-10 days	150-160	Mod-severe
4	**Ciprofloxacin**	51.2	oral	35	200 mg 7-10 days	21-35	mild

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

Thus taking consideration of cost, route of administration and side effects gatifloxacin is drug of choice.

DISCUSSION

This experiment was carried out to study the susceptibility of the bacterial isolates *Klebsiella pneumoniae (K. pneumoniae)* collected from sputum of RTI patients toward different 24 antibiotics. The percentages of susceptibility of *K. pneumoniae* isolates to the antibiotics which are commonly used to treat *K. pneumoniae* infections as shown in Table 2.

In the present study, the most predictable and primary etiological bacteria found to be involved in RTI are *K. pneumoniae* followed by *Pseudomonas*. Present study showed that *K. pneumoniae* is sensitive for only few antibiotics namelyamikacin, gatifloxacin and chloramphenicol. Antimicrobial sensitivity was tested for 24 antimicrobials for *K. pneumoniae* infection and most of them antimicrobials were found to be multidrug resistant. Out of 24 antibiotics tested, *K. pneumoniae* is showing sensitivity more than 50 % only to 4 (20%)antimicrobials and remaining 20 (80%) are showing less than 50 % sensitivity. *pneumoniae*is least sensitive to ticarcillin/ clavulinic acid, amoxicillin/clavulinic acid and polymixin-B. In situation of multidrug resistant 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 present study, it has been observed that *K. pneumoniae* showed low resistance to older drugs like chloramphenicol. It indicates that routine exposure of bacteria only to newly developed antibiotics eliminated resistance against older out of use antibiotics and present bacterial strains have grown sensitive to these outdated agents.

In present study, *K. pneumoniae* showed least resistance to amikacin. Aminoglycosides 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 could mean that there is a possibility of sensitivity to other aminoglycosides such as gentamycin, streptomycin, neomycin and kanamycin. However, this is not totally certain as each of the aminoglycosides have a slightly different mechanism of resistance due to their different aminoglycoside modifying enzymes chromosomal mutation e.g. streptomycin and impermeability of membranes [14].

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 [15]. Another mechanism is associated with penicillin-binding protein 2a (PBP2a), encoded by mecA2. Another gene involved in penicillin resistance in staphylococci is blaze which encodes β-lactamase [16].

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 betalactamases (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 harbored by *K. pneumoniae*[17]. Epidemic and endemic nosocomialinfections caused by ESBL producing *K. pneumoniae* represent a persistent problem in many parts of theworld, especially in ICUs [18-19]. Early identification of agent, therefore, is important for timely management of patients.

K. pneumoniae has been associated with different types of infections and one of the important aspects o f*K. pneumoniae* 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 resulting high morbidity and mortality. TEM- and SHV type ESBL producing *K. pneumoniae* were extensively reported worldwide after it was first identified in enterobacterial isolates from India [20]. The high prevalence of these drug resistant strains has further necessitated the requirement of a rapid and accurate identification system for *K. pneumoniae*.

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[21]. Moreover, uses of broad spectrum antibiotics, insufficient aseptic condition and technique with inadequate control of infections spread had aggravated this problem.

In vitro sensitivity is an important factor yet other factors given below should also be seriously considered in selecting the antimicrobial agents for an infection. For example cost of drugs for complete treatment, route of administration (oral, parenteral etc.), age (if the patient is neonate chloramphenicol is contraindicated) and pregnancy (tetracyclines are contraindicated). Other factors like allergic reactions to drugs like beta lactam antibiotic, kinetics of drugs and its concentration at the target site and mode and frequency of administration, bactericidal or bacteriostatic, efficacy/safety ratio, immunological status of the patient, ADR should also be considered[22].

CONCLUSIONS

Selection of drug of choice in any condition especially in infective diseases is not easy. We have to take into consideration the efficacy, safety, cost, pharmacokinetic, pharmacogenetics, convenience of administration and many other factors. In case of infectious diseases; we have to pay attention to microbial sensitivity and resistance pattern to various antimicrobials. The sensitivity pattern cannot be the sole criteria. Because it is done in vitro and it fails to take into account the immunological status of the patient and clinical condition of the patient.

An attempt has been made in this study to recognize the most common bacterial agent of infection in Surendranagar area and to record the antibiogram of the bacteria in this area. An attempt was again made to recognize the probable drug of choice based on antibiogram and some of the other factors namely the cost of treatment, mode of administration and adverse drug reactions. *K. pneumoniae* is most sensitive for amikacin followed by gatifloxacin, chloramphenicol, cefipime, ciprofloxacin and cefoperazone. Considering the antibiotic susceptibility testing, cost, convenience of administration, adverse drug reactions and many other factors amikacin should to be preferred followed by gatifloxacin, chloramphenicol for *K. pneumoniae* infection.

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