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ANTIMICROBIAL SENSITIVITY PATTERN OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM SPUTUM AND ISSUES RELATED TO THE RATIONALSELECTION 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 rational use of the existing and newer antimicrobial agents.. The present study is undertaken to prepare local antibiogram of *Pseudomonas aeruginosa* isolated from sputum and to discuss general issues related to antimicrobials use.Total 310 sputum samples were collected and tested bacteriologically using standard procedures. Culture positivity of urine samples was found to be 66.7 %. The most common pathogens were *K. pneumoniae* (40.2 %) followed by *Pseudomonas aeruginosa* (28.3%), *E. coli* (14.1%), *Staphylococci* (12.5%) and others (4.9 %). Antimicrobial susceptibility testing was done by disk diffusion method described by Kirby-Bauer (1961). *Pseudomonas aeruginosa* is most sensitive for gatifloxacin, cefoperazone, amikacin, cefipime, ceftazidime, norfloxacin, ciprofloxacin and ofloxacin.Considering the antibiotic susceptibility testing, cost, side effects and many other factors, gatifloxacin should be preferred for *Pseudomonas aeruginosa* infection for RTI.

KEYWORDS: Respiratory tract infection, sputum, *Pseudomonas aeruginosa*, antibiotic susceptibility testing, antimicrobial resistance, rational selection of antimicrobials.

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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]. In general practice bronchitis and pneumonia were most common lower respiratory tract infections and were related to considerable mortality and morbidity worldwide[4]. According to statistics 4.4% of hospital admissions and 6% of general practitionerconsultations were related to lower respiratory tract infections[5]. A wide variety of antimicrobial agents with anti-pseudomonal activity along with advancement in medical and surgical care has been developed but *Pseudomonas aeruginosa* causing life threatening infections continue to cause complications in hospitals acquired infection[6]. *Pseudomonas aeruginosa* is a Gram negative aerobic rod belongs to family Pseudomonadaceae. It became considered as an opportunistic pathogens and a major cause of nosocomial infections. It was also considered as most challenging pathogen globally because of its high rate of resistance to antimicrobial agents[7]. The *Pseudomonas aeruginosa* had very minimal nutritional requirement that expedited its growth in hospital environment[8-9]. *Pseudomonas aeruginosa* displayed resistance to multiple antimicrobial agents and only few antibiotics found to be effective against Pseudomonas aeruginosa[10]. A high resistance pattern of *Pseudomonas aeruginosa* measured as cause of higher mortality rate by Pseudomanal infections[11].

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 *Pseudomonas aeruginosa* and the development of multidrug-resistant strains that produce extended–spectrum beta-lactamase (ESBL). Epidemic strains of cephalosporin resistant *Pseudomonas aeruginosa* have been associated with increased morbidity and mortality in hospitalized patients[12].

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[13]. The microbiology laboratory plays a central role in the decision to choose a particular antimicrobial agent 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[14]. Different geographical locations and hospital environments showed variation in susceptibility pattern of *Pseudomonas aeruginosa* isolate therefore idiosyncrasy of isolate susceptibility pattern required for chemotherapeutic approach of *pseudomonal* infections for better achievement of results[15]. The aim and objective of the present study was to find out the prevalence and antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated from sputum and to discuss issue related to rational selection of antimicrobials in Surendranagar, Gujarat area.

MATERIALS AND METHODS

Sample collection

In the present study, 310 sputum samples from were processed in Department of Microbiology from inpatient & outpatient department of C.U.

Shah Medical College & Hospital Surendranagar; from period 1 July 2007 to 31st June 2008.

Biochemical characterization sample collection

The samples were transferred to microbiology laboratory and were analyzed within 30 min to 1 hour of collection. Nutrient agar, MacConkey agar and blood agar (Oxoid UK) used for streaking of sample and then incubated at 37°C for 24 hours as described by Cheesborough (Cheesborough2002)[16].

After incubation *Pseudomonas* isolation agar media (Oxoid UK) used for sub-culturing of isolate obtained. The pure isolates of *Pseudomonas aeruginosa* were transferred to 1% nutrient agar slant and stored in the refrigerator at 4 ± 1°C. Different identification tests were performed on suspected *Pseudomonas aeruginosa* and were characterized and identified i.e. Gram-stain, colonial morphology, positive oxidase reaction, production of pyocyanin on Mueller-Hinton agar (Oxoid UK), citrate utilization and growth at 42°C.

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[17]. 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[18]. Antibiogram for *Pseudomonas aeruginosa* was developed from antibiotic sensitivity testing then on the basis of antibiotic sensitivity, cost effectiveness and ADR profile, appropriate antibiotic for treatment of *Pseudomonas aeruginosa*, isolated from different sputum samples was achieved.

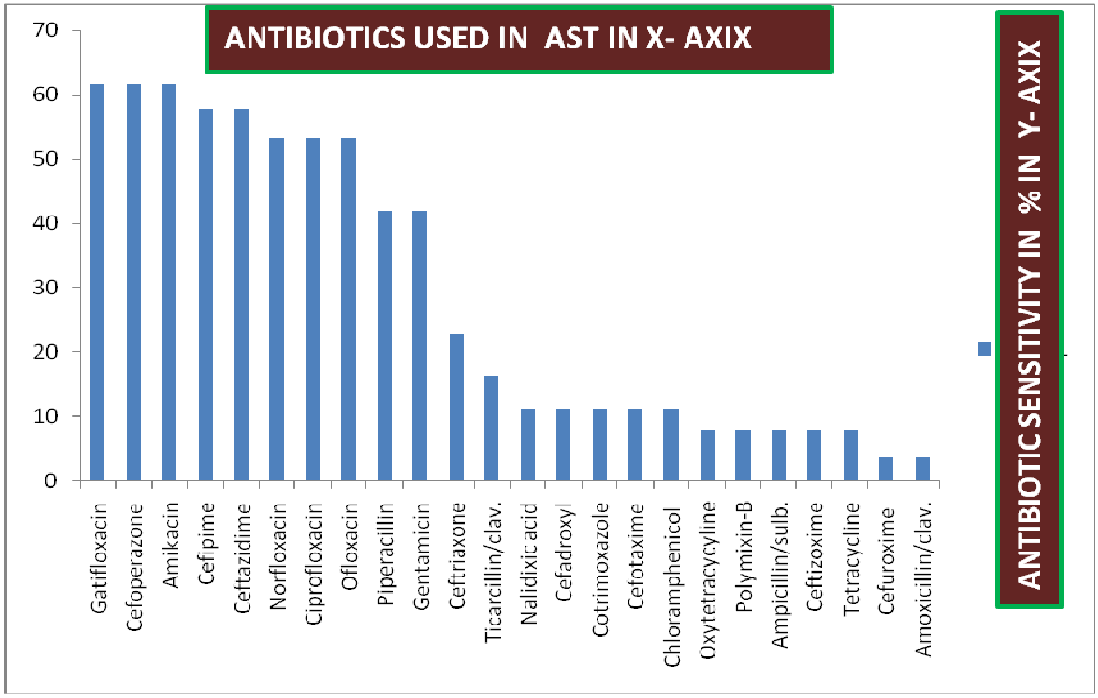
RESULTS

In the present study, 310 sputum samples from were processed in Department of Microbiology from inpatient & outpatient department of C.U. Shah Medical College & Hospital Surendranagar; from period 1st March 2007 to 29th Feb 2008. Out of all, 66.7% clinical isolates were recovered from sputum samples, the most common pathogens were *K. pneumoniae* (40.2%) followed by *Pseudomonas aeruginosa* (28.3%), *E. coli* (14.1%), *Staphylococci* (12.5%) and others (4.9%).

Table 1
Antibiotic Sensitivity of Pseudomonas

Antibiotics	**Sensitivity in %**	Resistance	Antibiotics	**Sensitivity in %**	Resistance
Gatifloxacin	**61.5**	38.5	Nalidixic acid	11.2	88.8
Cefoperazone	**61.5**	38.5	Cefadroxyl	11.2	88.8
Amikacin	**61.5**	38.5	Cotrimoxazole	11.2	88.8
Cefipime	**57.7**	44.3	Cefotaxime	11.2	88.8
Ceftazidime	**57.7**	44.3	Chloramphenicol	11.2	88.8
Norfloxacin	**53.2**	46.8	Oxytetracycyline	7.9	92.1
Ciprofloxacin	**53.2**	46.8	Polymixin-B	7.9	92.1
Ofloxacin	**53.2**	46.8	Ampicillin/sulb.	7.9	92.1
Piperacillin	41.9	58.1	Ceftizoxime	7.9	92.1
Gentamicin	41.9	58.1	Tetracycline	7.9	92.1
Ceftriaxone	22.9	77.1	Cefuroxime	3.8	96.2
Ticarcillin/clav.	16.3	83.7	Amoxicillin/clav.	3.8	96.2

FIGURE -1
Antibiotic Sensitivity of Pseudomonas



In above table we observe that *Pseudomonas aeruginosa* is most sensitive for gatifloxacin, cefoperazone, amikacin, cefipime, ceftazidime, norfloxacin, ciprofloxacin and ofloxacin. *Pseudomonas aeruginosa* is least sensitive for cefuroxime oxytetracycyline, polymixin-B and ampicillin/sulbactam, ceftizoxime, tetracycline and amoxicillin/clavulinic acid.

Table-2
Antibiotic of choice for Pseudomonas aeruginosa if isolated from sputum

Sr. No.	Name of drug	% Sensi.	Route of Adm.	Price	Total duration of treatment	Total cost For treatment	ADR/ Toxicity of drug
1	Gatifloxacin	61.5	Oral/ IV	50 Rs/ 10 tab	500 mg o.d. ×7-10 days	35-50 Rs	Mild
2	Cefoperazone	61.5	IV	310 Rs/ 1 gm vial	1 gm b.d. 5-7 days	3100-4340 Rs	Mild
3	Amikacin	57.8	IV/IM	10 Rs /500 mg vial	15mg/kg in 3 divided doses for 5 days	90-100 Rs	Mild –mod.
4	Ceftazidime	57.8	IV	355 Rs /vial	1 gm t.d.s. for 5 days	5325 Rs	Mild
5	Norfloxacin	53.8	ORAL	35 Rs/ 10 tab	400mg b.d. for 7-10 day	50- 70 Rs	Mild
6	Ciprofloxacin	53.8	Oral/IV	45 Rs/ 10 tab	500 b.d. for 7-10 day	63-90 Rs	Mild

Abbr. Sr. No.- Serial Number, IV- Intravenous, IM-Intramuscular, Adm.- Administration, Sensi.- Sensitivity, ADR- Adverse Drug Reaction, o.d.- once a day, b.d.- twice a day, t.d.s.- thrice a day, Rs- Rupees.

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

DISCUSSION

The main objective of this study was to investigate epidemiological data of *Pseudomonas aeruginosa* strains in lower respiratory tract infection patients and to determine the antimicrobial resistance pattern of bacteria against some commonly used antibiotics. The predominance of *Pseudomonas aeruginosa* resistance considered as serious problem in many countries[19-22]. It was also reported that *Pseudomonas aeruginosa* is one of the most common nosocomial pathogen and a leading cause of nosocomial respiratory tract infection[8, 9]. This experiment was carried out to study the susceptibility of the bacterial isolates *Pseudomonas aeruginosa* collected from sputum of RTI patients toward different 24 antibiotics. The percentages of susceptibility of *Pseudomonas aeruginosa*to the antibiotics which are commonly used to treat *Pseudomonas aeruginosa* infections are shown in Table 2 and figure1. In the present study, the most predictable and primary etiological bacteria found to be involved in RTI are *Klebsiella pneumoniae*followed by *Pseudomonas aeruginosa.*Out of 24 antimicrobials tested, *Pseudomonas aeruginosa* is showing maximum sensitivity (61.5%) only to three antimicrobials; more than 50% only to eight antimicrobials and remaining sixteen antimicrobials are showing less than 50% sensitivity. Present study showed that *Pseudomonas aeruginosa* is sensitive for only few antibiotics namely gatifloxacin, cefoperazone, amikacin, cefipime, ceftazidime, norfloxacin, ciprofloxacin and ofloxacin. *Pseudomonas aeruginosa* infection is found to be multidrug resistant. *Pseudomonas aeruginosa* is showing ≈ 90% resistance to 50% of antibiotics out of 24 antibiotics tested namely nalidixic acid, cefadroxyl, cotrimoxazole, cefotaxime, chloramphenicol, oxytetracycyline, polymixin-B, ampicillin/sulbactam, ceftizoxime, tetracycline and amoxicillin/ clavulinic acid. 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 the present study, *Pseudomonas aeruginosa* showed least resistance to amikacin. Aminoglycosides are proposed to be an alternative and better treatment of *Pseudomonas aeruginosa* infection in this part of the country. Furthermore, sensitivity of *Pseudomonas aeruginosa* to amikacin could

mean that there is a possibility of sensitivity to other aminoglycosides such as 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[23].

Overall resistance to third generation cephalosporins was high on account of the production of extended spectrum β-lactamases (ESBLs) by the *Pseudomonas aeruginosa*. 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[24]. 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-lactamase2 [25]. 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 *Pseudomonas aeruginosa*[26]. Epidemic and endemic nosocomial infections caused by ESBL producing *Pseudomonas aeruginosa* represent a persistent problem in many parts of the world, especially in ICU[27, 28]. Early identification of agent, therefore, is important for timely management of patients. 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[29]. 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[30].

CONCLUSION

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 in patient of respiratory tract 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. In present study it is found that *Pseudomonas aeruginosa* is most sensitive for gatifloxacin, cefoperazone, amikacin, cefipime, ceftazidime, norfloxacin, ciprofloxacin and ofloxacin. Considering the antibiotic susceptibility testing, cost, convenience of administration, adverse drug reactions and many other factors gatifloxacin should to be preferred followed by

amikacin cefipime, ceftazidime, norfloxacin, ciprofloxacin and ofloxacin for *Pseudomonas aeruginosa* infection.

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