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STUDY OF ANTIBIOTIC SUSCEPTIBILITY TEST OF MODERN GENERATION OF DRUGS AGAINST UPPER RESPIRATORY TRACT PATHOGENS

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ABSTRACT

Nasal infection or sinusitis is an inflammation of nasal passages caused by both viral and bacteriological pathogens. Antimicrobial resistance has universally recognized as growing problem concern about suitable therapy for nasal infection. The study was aimed at determining the prevalence and antimicrobial susceptibility against nasal infecting microorganisms. 50 clinical samples were taken from OPD of GMC Hospital, Bhopal (MP), India. Of the samples analyzed, 47 bacterial strains were isolated out of which 29 strains were of Gram positive bacteria (8 strains were of *Staphylococcus aureus,* 6 of *Staphylococcus epidermidis*, 7 of *Streptococcus pneumoniae* and 8 of *Corynebacterium diptheriae*) and 18 strains were of Gram negative bacteria (8 of *Escherichia coli,* 6 of *Pseudomonas aeruginosa* and 4 of *Neisseria meningitidis*). Antimicrobial susceptibility assay was performed by disc diffusion method according to the reference criteria of clinical and laboratory standard institute guidelines. In the present study antibiotic susceptibility pattern results showed maximum level of resistance in gram positive strains *S. aureus* 8 (100%), *S. epidermidis* 6 (100%) and *C. diptheriae* (8 (100%) against penicillin, *S. aureus* 8 (100%), *S. epidermidis* 6 (100%) and *S. pneumoniae* 7 (100%) were resistant to Cefuroxime, *S. aureus* 7 (87.5%), *S. epidermidis* 6 (100%), *S. pneumoniae* 7 (100%) and *C. diptheriae* (8 (100%) were resistant to erythromycin and azithromycin whereas, rest of gram positive strains showed satisfactory antibiotic susceptibility against chloramphenical, cefazolin, cephalexin, ciprofloxacin, ofloxacin and tetracyclin. Similarly for gram negative strains multi-drug resistance was observed in 8 (100%) isolates of *E. coli* against aztreonam, cefdinir, cefixime, cefotaxime, ceftriaxone, ceftazidime, cefuroxime, ciprofloxacin, nalidixic acid and ofloxacin, *P. aeruginosa* 6 (100%) were resistant to aztreonam, cefdinir, cefixime, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, nalidixic acid and ofloxacin, *N. meningitidis* 4 (100%) were resistant to aztreonam, cefdinir, cefixime, cefotaxime, ceftazidime, nalidixic acid and ofloxacin whereas rest of gram negative strains showed moderate susceptible against amikacin, cefuroxime and ciprofloxacin. Hence we concluded that the efficacy of cefazolin, cephalexin, chloramphenical, tetracycline, amikacin and ciprofloxacin was recorded higher than other antibiotics tested against nasal infection causing pathogens.

INTRODUCTION: Nasal infection is usually defined as an acute bacterial infection involving the mucosal surfaces of the paranasal sinuses and nasal cavity [1-3]. Sometimes referred to as sinusitis or rhinosinusitis is an ailment marked by an inflammation of the paranasal sinuses, often as a consequence of a bacterial infection. Infections with the formation of a bacterial biofilm account for many cases of antibiotic-resistant chronic sinusitis. When a biofilm forms on an infection, treatment becomes very complicated, since the bacteria inside it exists in a state that renders it largely immune to antibiotics. The American Academy of Allergy, Asthma and Immunology reports that sinusitis is one of the leading forms of chronic disease, with an estimated 18 million cases and at least 30 million courses of antibiotics in the United States per year.

The nares (nostrils) are always heavily colonized, predominantly with *Staphylococcus epidermidis* and *Corynebacteria* spp. and often (in about 20% of the general population) with *Staphylococcus aureus*, this being the main carrier site of this important pathogen. The healthy sinuses, in contrast are sterile. Bacterial pathogens in acute sinusitis are *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis*. These particular bacteria are frequently referred to as "respiratory pathogens".

Other potential organisms include *Streptococcus pyogenes*, *S. aureus* as well as mixed anaerobic bacteria (*Peptostreptococcus, Fusobacterium, Bacteroides, Prevotella*). *S. aureus* is a member of commensal microflora and readily colonizes the anterior nares in humans. Many infections caused by *S. aureus* occur in persons with prior nasal carriage[4] and this carriage is an important risk factor for nosocomial *S. aureus* infection in patients undergoing surgery, hemodialysis, implantation of intravascular devices, and among HIV-infected patients [5].

Typical organisms isolated in chronic sinusitis include the respiratory pathogens such as *S. epidermidis*, *S. aureus*, anaerobes and gram-negative rods. *Pseudomonas aeruginosa* may also be present [6-9]. To further complicate matters, chronic sinusitis is often a polymicrobial disease with cultures usually growing multiple pathogens [6,8].

The relationship between antibiotic use and resistance is complex a population genetics study demonstrated that the volume of drug use can influence the selection pressure for antibiotic resistance, but a quantitative relationship between these two factors was not demonstrated. Using an antimicrobial drug for any infection, in any dose and over any period of time forces microbes either to adapt or to die; microbes that adapt carry genes for drug resistance that are then passed on. But when an antimicrobial is used in appropriately for too short a time, at too low a dose, at inadequate potency or for the wrong disease, microbes are more likely to develop resistance to that drug.

The reduction in antibiotic resistance can only occur following a significant reduction in antibiotic use. It has been argued that the time require for a drop in the prevalence of antibiotic resistance to occur will be more than the time required for resistance to develop under a constant selective pressure. To overcome the arising effects of nasal infection researches have been performing worldwide. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain.

Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic and develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. The aim of the study was to investigate the antimicrobial susceptibility against nasal infection causing microorganisms.

MATERIALS AND METHODS:

Bacterial Isolates and Sampling Procedure: A total of fifty samples of sinus belonging to different nasal infection were taken from OPD of Gandhi Medical College, Bhopal (MP), India. Nasal sample were collected with sterile cotton swabs from the actual infection site aseptically into the sterile container containing normal saline. A nasal swab will capture the causative organism in most cases and the culture will allow the specific organism to be grown in the microbiology laboratory under certain conditions. Samples were taken to the laboratory in cold condition within 4 hr for further microbiological analysis.

Bacterial Isolation and Identification: The samples were inoculated on nutrient agar and MacConkey agar media plate and incubated at 37°C for 24 hr. The colonies of isolated organism have been sub culture on nutrient agar plate and pure culture were obtained in various selective media such as Mannitol Salt agar, Blood agar and Chocolate agar and further identified by biochemical tests for confirmation of bacteria. For the confirmation of the isolated bacteria and their species specific biochemical tests such as IMViC test, catalase test, coagulase test, urease test, oxidase test, bile solubility test, Deoxyribonuclease (DNAse) test and carbohydrate fermentation test were performed.

S. aureus, was identified by the positive catalase, from IMViC-MR positive and acid-gas production by carbohydrate fermentation test and other biochemical characters. *S. epidermidis*, isolated on Mannitol Salt agar and identified by the positive catalase, IMViC -MR and carbohydrate fermentation tests. *S. pneumoniae,* was isolated on Streptococcus selection agar and identified positive for IMViC-VP and bile solubility test. *Corynebacterium diptheriae*, isolated on Dextrose Proteose Peptone Agar (DPPA), showed positive results of catalase and carbohydrate fermentation shown in **figure 1**.

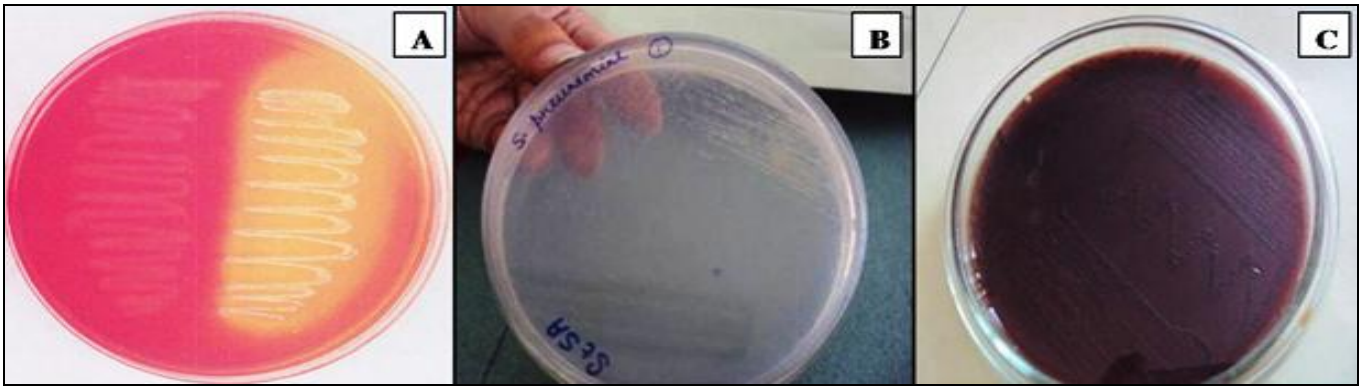

FIG. 1: PURE BACTERIAL CULTURES OF GRAM POSITIVE BACTERIA FROM VARIOUS SITES OF NASAL INFECTIONS (A) *S. EPIDERMIDIS* AND *S. AUREUS* ON MANNITOL SALT AGAR; (B) *S. PNEUMONIA* ON STREPTOCOCCUS SELECTION AGAR; (C) *C. DIPHTHERIA* ON DEXTROSE PROTEOSE PEPTONE AGAR

Escherichia coli, isolated on Nutrient agar media and identified positive for IMViC-Indole, MR, catalase and carbohydrate fermentation. *P. aeruginosa,* isolated on Pseudomonas agar base and identified positive for catalase, oxidase and citrate utilization. *Neisseria meningitidis*, isolated on Chocolate Agar, showed positive results for catalase, oxidase and carbohydrate fermentation shown in **figure 2**.

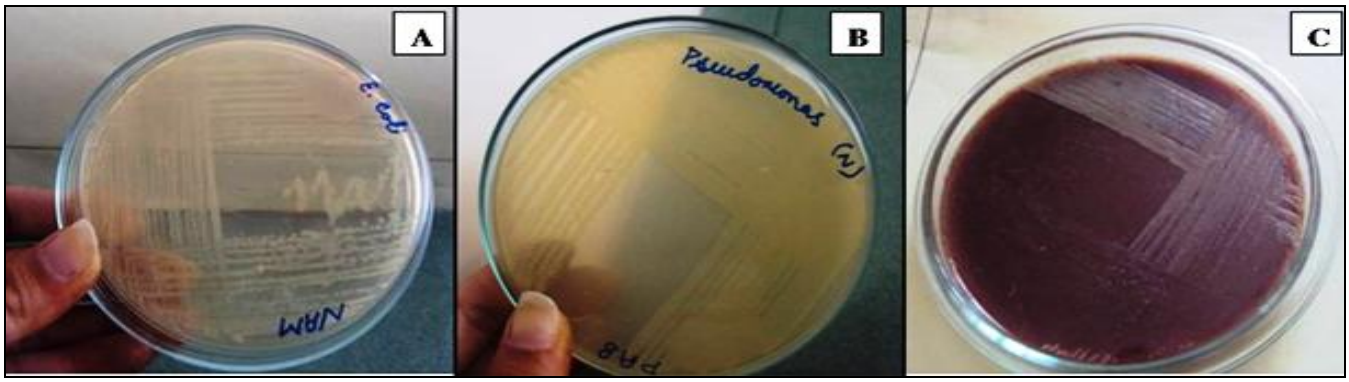

FIG. 2: PURE BACTERIAL CULTURES OF GRAM NEGATIVE BACTERIA FROM VARIOUS SITES OF NASAL INFECTIONS (A) *E. COLI* ON NUTRIENT AGAR MEDIA; (B) *P. AERUGINOSA* ON PSEUDOMONAS AGAR BASE; (C) *N. MENINGITIDIS* ON CHOCLATE AGAR

Antibiotic susceptibility assay: Antibiotic susceptibility testing of the isolates was carried out by Kirby Bauer's assay [10]. Briefly, the culture medium used for the antibiotic susceptibility was Mueller Hinton Agar (MHA). Inoculation in the nutrient broth for 24 hr for antibiotic sensitivity assay was performed by the reference criteria of clinical and laboratory standard institute guidelines [11]. Broth culture of test organisms was homogeneously was spread on the surface with the help of glass spreader on Mueller-Hinton agar (MHA) plate and then the antibiotic discs were prepared using different antibiotics (Amoxicillin,

Azithromycin, Cefazolin, Cefuroxime, Cephalexin, Chloramphenicol, Ciprofloxacin, Erythromycin, Ofloxacin, Penicillin and Tetracycline were used for gram positive bacteria while Amikacin, Aztreonam, Cefdinir, Cefixime, Cefotaxime, Ceftazidime, Ceftriaxone, Cefuroxime, Ciprofloxacin, Nalidixic acid and Ofloxacin were used for gram negative bacteria) and placed immediately with a sterile forceps. Susceptibility test plate was then incubated by keeping them inverted in incubator at 37^{o}C for 24 hr, results were interpreted as per the standard literature provided with the commercial discs.

RESULTS: The present study was carried out on 50 samples of sinus belonging to different nasal infection. Among 50 clinical samples, 47 bacterial strains were isolated out of which 29 strains were of Gram positive bacteria (8 strains were of *S. aureus,* 6 of *S. epidermidis*, 7 of *S. pneumoniae* and 8 of *C. diptheriae*) and 18 strains were of Gram negative bacteria (8 of *E. coli,* 6 of *P. aeruginosa* and 4 of *N. meningitidis*). The isolated organisms were characterized on the basis of morphological, cultural and biochemical characteristics.

Antibiotic resistance pattern of 29 isolates of Gram positive strains is shown in **figure 3**. Resistance to commonly used antibiotics against *S. aureus* isolates, penicillin with cefuroxime 8 (100%), 7 (87.5%) resistant to amoxicillin with erythromycin, ofloxacin and azithromycin, cefazolin 6 (75%), cephalexin 5 (62.5%), ciprofloxacin 4 (50%). Whereas, higher susceptibility was noted to chloramphenicol with tetracycline 6 (75%). *S. epidermidis* isolates resistance to commonly used antibiotics shown, penicillin with cefuroxime, erythromycin and azithromycin 6 (100%), ofloxacin 4 (66.6%), tetracycline 3 (50%). Whereas, higher susceptibility was noted to cefazolin with ciprofloxacin 6 (100%), cephalexin with chloramhenicol 5 (83.3%).

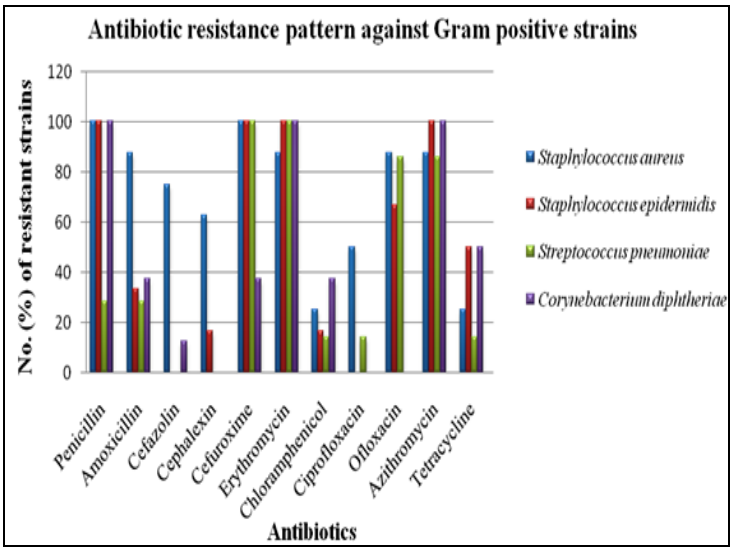

FIG. 3: PERCENTAGE OF RESISTANCE PATTERN OF GRAM POSITIVE ISOLATES FROM VARIOUS SITES OF NASAL INFECTIONS

S. pneumoniae isolates resistance to commonly used antibiotics shown, cefuroxime with erythromycin 7 (100%), ofloxacin with azithromycin 6 (85.7%), penicillin with amoxicillin 2 (28.5%). Whereas, higher susceptibility was noted to cefazolin with cephalexin 7 (100%), chloramphenicol with ciprofloxacin and tetracycline 6 (85.7%). *C. diptheriae* isolates resistance to commonly used antibiotics shown, penicillin with erythromycin and azithromycin 8 (100%), tetracycline 4 (50%), amoxicillin with cefuroxime and chloramphenicol 3 (37.5%). Whereas, higher susceptibility was noted to cephalexin with ciprofloxacin and ofloxacin 8 (100%), cefazolin 7 (87.5%) that are shown in **Table 1**.

TABLE 1: ANTIBIOTIC RESISTANCE PATTERN AGAINST GRAM POSITIVE STRAINS

Antibiotics (Concentration in mcg)	No. (%) of resistant strains (n=29)			
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Streptococcus pneumoniaeee*	*Corynebacterium diptheriaee*
Penicillin (10)	8 (100%)	6 (100%)	2 (28.5%)	8 (100%)
Amoxicillin (10)	7 (87.5%)	2 (33.3%)	2 (28.5%)	3 (37.5%)
Cefazolin (30)	6 (75%)	0 (0%)	0 (0%)	1 (12.5%)
Cephalexin (30)	5 (62.5%)	1 (16.6%)	0 (0%)	0 (0%)
Cefuroxime (30)	8 (100%)	6(100%)	7 (100%)	3 (37.5%)
Erythromycin (15)	7 (87.5%)	6(100%)	7 (100%)	8 (100%)
Chloramphenicol (30)	2 (25%)	1 (16.6%)	1 (14.2%)	3 (37.5%)
Ciprofloxacin (5)	4 (50%)	0 (0%)	1 (14.2%)	0 (0%)
Ofloxacin (5)	7 (87.5%)	4 (66.6%)	6 (85.7%)	0 (0%)
Azithromycin (15)	7 (87.5%)	6(100%)	6 (85.7%)	8 (100%)
Tetracycline (30)	2 (25%)	3 (50%)	1 (14.2%)	4 (50%)

The antibiotic resistance pattern of 18 isolates of Gram negative strains is shown in **figure 4**. Multi-drug resistance was observed in 8 (100%) isolates of *E. coli* against aztreonam, cefdinir, cefixime, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, nalidixic acid and ofloxacin except for 4 (50%) *E. coli* isolates which were susceptible to amikacin. Similarly multi-drug resistance was also observed in 6 (100%) *P. aeruginosa* isolates against aztreonam, cefdinir, cefixime, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, nalidixic acid and ofloxacin.

Whereas, higher susceptibility was noted to ciprofloxacin 6 (100%), amikacin 3 (50%). A multi-drug resistance was also seen in 4 (100%) *N. meningitides* isolates against aztreonam, cefdinir, cefixime, ceftazidime, cefotaxime, nalidixic acid and ofloxacin, 3 (75%) *N. meningitidis* were resistant to ceftriaxone and amikacin. Whereas, higher susceptibility was noted to cefuroxime 2 (50%) respectively that are shown in **Table 2**.

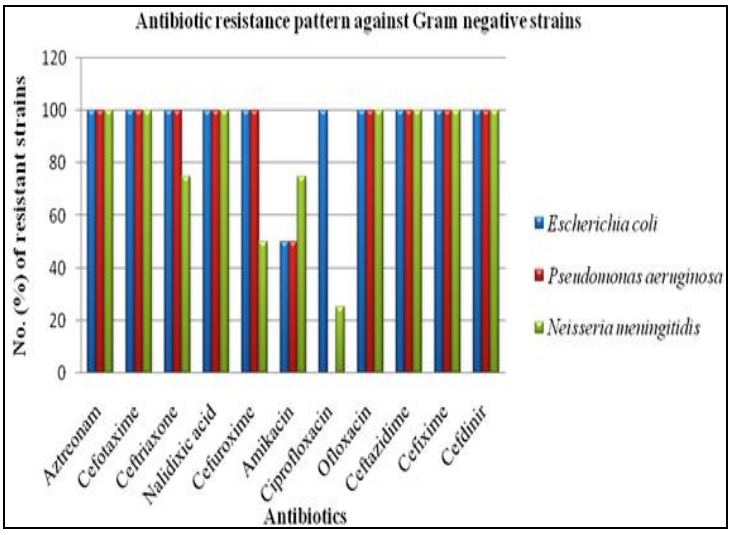

FIG. 4: PERCENTAGE OF RESISTANCE PATTERN OF GRAM NEGATIVE ISOLATES FROM VARIOUS SITES OF NASAL INFECTIONS

TABLE 2: ANTIBIOTIC RESISTANCE PATTERN AGAINST GRAM NEGATIVE STRAINS

Antibiotics (Concentration in mcg)	No. (%) of resistant strains (n=18)		
	Escherichia coli	*Pseudomonas aeruginosa*	*Neisseria meningitidis*
Aztreonam (30)	8 (100%)	6 (100%)	4 (100%)
Cefotaxime (30)	8 (100%)	6 (100%)	4 (100%)
Ceftriaxone (30)	8 (100%)	6 (100%)	3 (75%)
Nalidixic acid (30)	8 (100%)	6 (100%)	4 (100%)
Cefuroxime (30)	8 (100%)	6 (100%)	2 (50%)
Amikacin (30)	4 (50%)	3 (50%)	3 (75%)
Ciprofloxacin (5)	8 (100%)	0 (0%)	1 (25%)
Ofloxacin (5)	8 (100%)	6 (100%)	4 (100%)
Ceftazidime (30)	8 (100%)	6 (100%)	4 (100%)
Cefixime (5)	8 (100%)	6 (100%)	4 (100%)
Cefdinir (5)	8 (100%)	6 (100%)	4 (100%)

DISCUSSION: Antimicrobial resistance among bacterial pathogens is a worldwide issue. By the increasing of drug resistance nasal bacterial strains are harmful to our health. The antimicrobial susceptibility pattern of common pathogenic bacteria is essential to guide empirical and pathogen specific therapy. To ensure an efficient and adequate therapy it is necessary to identify and treat the focus of infection. It was found that Gram positive and Gram negative bacterial pathogens are a common cause of infection and the prevalence and rates of resistance to existing antimicrobial agents are increasing. Resistance towards antibiotics is associated with an increase in disease severity, which increases period of hospitalization, high mortality and increasing treatment costs, including a need for use of alternative drugs.

The selection and spread of resistant organisms in developing countries, can led to complex socioeconomic behavioral antecedents and misuse of antibiotics by health professionals, unskilled practitioners, layman, poor drug quality and unhygienic conditions for spread of resistant bacteria, and inadequate surveillance [12, 13].

Our study showed a total number of 50 as Gram positive and Gram negative. All the strains of bacteria were subjected to biochemical tests and were aimed to study their drug susceptibility pattern. Most of the antibiotic tested showed potent activities against Gram positive isolates in which cephalexin and cefazolin were the most active antimicrobial agents whereas Gram negative isolates were highly susceptible to Amikacin.

Penicillin, cefuroxime and had the highest resistance to all the Gram positive strains. *S. aureus* resistance to penicillin is mediated by penicillase production, an enzyme that cleaves the β- lactam ring of the penicillin molecule, rendering the antibiotic ineffective. The prevalence of S. aureus nasal carriage among healthy adults ranges from approximately 20% to 30%, with higher prevalences in overcrowded populations [14, 15].

Erythromycin, azithromycin and ofloxacin showed intermediate resistance against all Gram positive stains. Whereas, the cephalosporin group of antibiotic cefazolin and cephalexin showed maximum sensitivity against Gram positive strains. This group of antibiotic interfere with the bacterial cell wall synthesis by blocking the cross linking of the peptide glycan units.

The antibiotic ciprofloxacin showed maximum sensitivity against *S. epidermidis, C. diptheriae, P. aeruginosa and N. meningitides* [16, 17]. This binds to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis. *P. aeruginosa* and *Staphylococci* are more commonly isolated from patients with chronic sinusitis. Therefore use of these antibiotics should be restricted to severe nasal infections, in order to avoid rapid emergence of resistant strains.

Most of the Gram negative strains were displayed by highest number of multi-drug resistance to most of the antibiotics except the amikacin. Amoxicillin and cephalexin are all β–lactam antibiotics that showed susceptibility against all Gram positive isolates. β–lactam antibiotics are a broad class of antibiotics, consisting of all antibiotic agents that contains a β–lactam nucleus in its molecular structure. β–lactam antibiotics works by inhibiting the cell wall synthesis. β–lactam antibiotics are bacteriocidal and act by inhibiting the synthesis of peptidoglycon layer of bacterial cell wall.

The final transpeptidation step in the synthesis of the peptidoglycon is facilitated by transpeptidase known as penicillin binding proteins (PBPs). β–lactam antibiotics are analogues to D-alanyl-D-alanine the terminal amino acid residue on the precursor NAM/NAG-peptide subunit of a nascent peptidoglycon layer. The production of β–lactamase by a bacterium does not necessarily rule out of all treatment options with β–lactam antibiotics. In some instance, β–lactam antibiotics may be co-administered with a β–lactamase inhibitor (clavulanic acid, tazobactam, sulbactam).

Erythromycin, a macrolide targeting the bacterial 50S ribosomal subunit, effectively inhibits protein synthesis [18].

Chloramphenicol is bacteriostatic (that is, it stops bacterial growth). It is a protein synthesis inhibitor (more specifically inhibiting protein chain elongation), causing inhibition of peptidyl transferase activity of the bacterial ribosome, binding to A2451 and A2452 residues in the 23S rRNA of the 50S ribosomal subunit, preventing peptide bond formation. There are three mechanisms of resistance to chloramphenicol: reduced membrane permeability, mutation of the 50S ribosomal subunit and elaboration of chloramphenicol acetyl transferase.

Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. It is a second-generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and of protein. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division.

Ofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class considered to be a second-generation fluoroquinolone.

The fluoroquinolones interfere with DNA replication by inhibiting an enzyme complex called DNA gyrase.

Tetracycline is a broad-spectrum polyketideantibiotic it is a protein synthesis inhibitor. Tetracycline binds to the 30S subunit of microbial ribosomes. They inhibit protein synthesis by blocking the attachment of charged aminoacyl-tRNA. Thus they prevent introduction of new amino acids to the nascent peptide chain. The action is usually inhibitory and reversible upon withdrawal of the drug. Resistance to the tetracyclines results from changes in permeability of the microbial cell envelope.

In order to reduce the development of drug resistance, combination therapies should be used that is achieved by giving separate drugs or by giving combination drugs which are dosage forms that contain more than one active ingredient.

CONCLUSION: We can conclude that knowledge of emerging pathogens and resistance profile is essential for treatment against nasal infections. Shorter duration of treatment and correct dosage of antibiotic therapy is recommended to reduce the selection pressure for resistant isolates.

Human body is perfect natural habitat for number of microorganisms like bacteria, fungi, yeast and some viruses which are termed as microflora or normal flora of a body. The nose is certainly a prominent organ for it sits right smack in the middle of our face. The nose is one of the body's first lines of defense against infection. It provides shelter for many microorganisms due to presence of all type of nutrients and environment. The frequency of the drug resistant bacteria has increased as compared to the previous years and it is important issue to be addressed.

This study demonstrates that the continued evolution and geographical variation in bacterial resistance, highlights the need of appropriate antimicrobial agents. There use will depend upon the adequate activity and local susceptibility profile. Effective infection control programs are essential to controlling and preventing nasal infections. These programs include a core of the infection control committee, infection control practitioner and individual employee actions.

In the future, issues of concern about the emergence of nosocomial infections, increasing antimicrobial resistance and the increase in morbidity, mortality and costs associated with these infections will drive the need for refinement of molecular approaches to aid in the diagnosis and epidemiologic analysis of nosocomial infections. The evaluation of hospital-associate infections will continue to rely on clinical infection surveillance as the first step to understanding disease epidemiology and management of infections. The most accurate assessment of epidemiologic relationships in a nosocomial setting is always accomplished by careful assessment of all available information.

Molecular testing will continue to be an essential tool, for the testing has proven to be cost-effective and medically needed. Molecular typing is a powerful tool in the armamentarium for combating the spread of problem microorganisms in the hospital environment. Thus, it is important that we seek to continually improve existing infection control policies and programs.

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