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Original Research Article

Antibiotic susceptibility pattern of organisms in chronic rhinosinusitis

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ABSTRACT

Background: Chronic rhinosinusitis is an ever increasing problem faced by all sectors of the population throughout the world. Various etiologic factors have been associated with the disease entity but the main concern throughout the world is the increasing antimicrobial resistance which is leading to less compliance and higher treatment cost.
Methods: A total of 60 cases and 60 controls were selected and nasal swabs were collected under endoscopic guidance from middle meatus and were sent for culture and sensitivity.
Results: The data collected showed that the most common pathogen isolated from the cases was methicillin resistant *Staphylococcus aureus* (MRSA) accounting for 58.33% followed by fungi and methicillin susceptible *Staphylococcus aureus* (MSSA). The antibiotic sensitivity showed that MRSA is having the most resistant pattern with only Vancomycin, Teicoplanin and Linezolid having 100% sensitivity followed by Rifampicin, Netilmicin and Clindamycin.
Conclusions: From this study we conclude that MRSA is the most common pathogen affecting the cases of chronic sinusitis and it shows a high degree of resistance towards antibiotics which is responsible for incurring higher cost of treatment and low compliance.

Keywords: Rhinosinusitis, Antibiotics, *Staphylococcus*, *H. influenzae*, Osteomeatal complex

INTRODUCTION

Chronic rhinosinusitis as the term suggest is the involvement of the nose and paranasal sinus by the disease and the chronicity is labelled by the persistence of the symptoms and signs for more than 12 weeks despite of treatment in form of antimicrobials, analgesics, antihistaminic, nasal decongestants and steroid spray. The recent literature considering the etiology of chronic sinusitis focuses mainly on the obstruction of the ostiomeatal complex with the infectious organisms considered as the secondary invaders but their role cannot be completely ruled out as they are mainly responsible for the chronicity of the disease process.

Many bacteria and fungi have been isolated and documented in literature, they are:

Staphylococcus aureus (both methicillin-susceptible S aureus [MSSA] and methicillin-resistant *S. aureus* [MRSA] strains).[1] Coagulase-negative *Staphylococci*, *H. influenza*, *M. catarrhalis*, *S. pneumonia*, *Streptococcus intermedius*, *Pseudomonas aeruginosa*, *Nocardia* species and anaerobic bacteria (*Peptostreptococcus, Prevotella, Porphyromonas, Bacteroides, Fusobacterium* species.[2,3]

Various fungal elements have also been isolated namely *Aspergillus species*, *Cryptococcus neoformans*, *Candida* species, *Sporothrix schenckii*, *Alternaria* species.[4]

Bacteria are notorious in the sense that they develop resistance to the antibiotics very rapidly, the over enthusiastic use and improper dosage recommended by untrained physicians and compliance of the patient are some of the reasons which have led to the rise in antimicrobial resistance and in this context World Health Organization has named antibiotic resistance as one of the three most important public health threats of the 21st century.[5]

The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are:

1. Drug inactivation or modification
2. Alteration of target or binding site
3. Alteration of metabolic pathway
4. Reduced drug accumulation

Antibiotic resistance can be a result of horizontal gene transfer.[6]

Hence, taking into consideration the antimicrobial resistance a prospective case control study was undertaken to determine the resistance pattern of organisms isolated from patients of chronic rhinosinusitis.

METHODS

The study was conducted in the Department of E.N.T from March 2016 to September 2016, after formal ethical approval from the Institutional Ethical committee. The cases of chronic rhinosinusitis were diagnosed on the criterias laid down by the European position paper on rhinosinusitis and nasal polyps 2012.[7]

Inclusion criteria

Patients attending the outpatient Department of E.N.T with signs and symptoms as per the European position paper on rhinosinusitis and nasal polyps 2012.

Exclusion criteria

Exclusion criteria were pregnant females, children below age of 2 years, immunocompromised individuals.

Under all aseptic precautions nasal swabs were taken from the secretions present in the middle meatus under endoscopic guidance and were sent for culture and sensitivity. The swabs were then subjected to inoculation on appropriate bacteriological media, including 10% sheep blood agar, chocolate agar, thioglycollate, Mac-Conkey agar media. The plates were incubated at 37 °C for 18-24 hours.

The primary identification of the organisms were made by colony morphology, Gram staining, catalase and coagulase tests. The final antibiotic sensitivity was obtained by disc-diffusion method and the isolated bacteria were subjected to susceptibility testing against the following:

Staphylococcus sp: Penicillin, Oxacillin, Cephalothin, Gentamicin, Netilmicin, Amikacin, Chloramphenicol, Tetracycline, Erythromycin, Co-trimoxazole, Clindamycin, Ofloxacin, Rifampicin, Vancomycin, Teicoplanin.

Gram negative bacilli: Ampicillin, Piperacillin, Cephalothin, Cefotaxime, Ceftazidime, Gentamicin, Netilmicin, Amikacin, Chloramphenicol, Tetracycline, Cotrimoxazole, Nalidixic Acid, Ciprofloxacin, Ofloxacin, Nitrofurantoin, Imipenem, Meropenem.

Streptococcus: Penicillin, Oxacillin, Ampicillin, Cefotaxime, Erythromycin, Chloramphenicol, Tetracycline, Vancomycin.

Haemophilus sp: Ampicillin, Amoxycillin/Clavulanic acid, Cefuroxime, Cefotaxime, Tetracycline, Erythromycin, Chloramphenicol.

The data obtained was analysed by the Open EPI info which is an online statistical analysis program under the aegis of Centre for Disease Control and Prevention.

RESULTS

Out of 60 cases 43 patients showed infestation of *Staphylococcus* species and 4 by *H. influenzae*, 12 patients showed growth of fungi and 1 showed no-growth, among controls, 16 patients showed growth of *Staphylococcus* species, 7 showed growth of *H. influenzae*, 5 showed the growth of fungi 32 showed no-growth.

Now taking into consideration the antibiotic sensitivity of the MRSA which constitutes 58.33% of all the isolated pathogens we determined that it has developed complete resistance against penicillin as tested in the samples collected from cases and controls, next in the list is Cotrimoxazole and Erythromycin to which it shows only 2.85% sensitivity. The sensitivity of the organisms increased with higher order antibiotics with Vancomycin, Teicoplanin and Linezolid having 100% sensitivity followed by Rifampicin, Netilmicin and Clindamycin. The sensitivity of other antimicrobial agents and their effectiveness in other *Staphylococcus* species has been summarized in Table 1.

Taking into consideration the *H. influenzae* the effectiveness of various antimicrobial agents has been depicted in Table 2.

Table 1: The sensitivity of antimicrobial agents and their effectiveness on *Staphylococcus* species.

Antimicrobial agents	Cases MRSA (n=35) (%)	Controls MRSA (n=10) (%)	Cases MSSA (n=6) (%)	Controls MSSA (n=2) (%)	Cases *S. epidermidis* (n=2) (%)	Controls *S. epidermidis* (n=4) (%)
Penicillin	0	0	0	0	50	50
Oxacillin	20	60	16.66	100.00	100	100
Cephalothin	37	60	50	100.00	100	100
Gentamicin	8.65	50	0	0	100	100
Netilmicin	46	80	33.33	50.00	100	100
Amikacin	25.70	90	100	100.00	100	100
Chloramphenicol	11.40	60	50	50.00	100	100
Tetracycline	14	30	83.33	50	100	50
Erythromycin	2.85	30	50	50	50	75
Co-trimoxazole	2.85	50	50	50	100	100
Clindamycin	42.85	100	100	100	100	100
Ofloxacin	34.28	80	100	100	100	100
Rifampicin	48.57	60	66.66	100	100	100
Vancomycin	100	100	100	100	100	100
Teicoplanin	100	100	100	100	100	100
Levofloxacin	38.33	100	100	100	100	100
Linezolid	100	100	100	100	100	100

Table 2: The sensitivity of antimicrobial agents and their effectiveness on *H. influenzae*.

Antimicrobial Agents	Cases *H. influenzae* (n=4) (%)	Controls *H. influenzae* (n=7) (%)
Ampicillin	0	28.57
Amoxycillin/Clavulanic acid	75	71.42
Cefuroxime	100	85.71
Cefotaxime	100	100
Tetracycline	50	57.14
Erythromycin	25	42.85
Chloramphenicol	50	42.85

Table 3: Antibiotic grouping on the basis of mechanism of action.

Cell wall synthesis	Penicillin
	Cephalosporins
	Oxacillin
	Vancomycin
	Teicoplanin
Protein synthesis inhibitors	Inhibit 30s Subunit
	○ Aminoglycosides (Gentamicin, Netilmicin)
	○ Tetracyclines
	○ Amikacin
	Inhibit 50s subunit
	○ Erythromycin
	○ Macrolides
	○ Chloramphenicol
	○ Clindamycin
	Linezolid
DNA synthesis inhibitors	Fluoroquinolones
RNA synthesis inhibitors	Rifampin
Folic acid synthesis inhibitors	Sulfonamides
	Trimethoprim

DISCUSSION

MRSA has been found to be the most prevalent organism in the whole study comprising 58.33% of all the isolates from cases, in a similar study Brook isolated MRSA from 60% of cases of chronic sinusitis and had a complete resistance to penicillin.[1,3,25] The antimicrobial agents which have been used to test the sensitivity against Staphylococcal species are grouped as shown in Table 3.

The resistance against these antimicrobial agents is attained by four basic mechanisms which are limiting uptake, modifying the target, drug inactivation and increased efflux. The acquisition of these mechanisms could be intrinsic (the bacteria have the gene which is needed to be activated) or extrinsic (bacteria acquires resistance by DNA transfer from resistant bacteria by plasmids, bacteriophages). The mechanism by which the staphylococcus species acquires resistance against the various groups of antimicrobial agents is as follows:

1. Affecting cell wall synthesis

The initial resistance to β-lactam antibiotics is by production of the β-lactamase enzyme within the cell which was acquired by a plasmid that contained the gene *blaZ.* Thereafter newer β-lactam drugs were produced which were resistant to the effect of β-lactamase, but in response the bacteria acquired *mecA* gene which led to the production of Penicillin binding protein 2 (PBP2) as a constituent of the cell wall and has less affinity for methicillin and most other β-lactam drugs.[8]

Various reports have also documented the resistance to Vancomycin which has led to the development of two types of strains i.e. Vancomycin-intermediate *S. aureus* (VISA) and Vancomycin-resistant *S. aureus* (VRSA). The mechanism of resistance in VISA has been documented to the thickening of the cell wall which impairs the penetration of Vancomycin through it but the exact gene responsible for it has not been determined.[9,10]

On the other hand the resistance in VRSA have been mediated by the acquisition of vanA gene from enterococci which cause modification in the peptidoglycan precursors and decreases the binding affinity for Vancomycin.[11,12]

2. Affecting the protein synthesis

Binding to the 30S ribosomal subunit

The mechanism of resistance for Aminoglycoside is by production of aminoglycoside-modifying enzyme (AME) which chemically modifies the drug mainly by the transfer of the acetyl group which in turn decreases the ability to bind to 30S ribosomal subunit. The production of AME has been related to the acquisition of aac, ant, and aph genes which are acquired via a plasmid.[13,14] Resistance against tetracycline has been acquired by two mechanisms firstly by active efflux because of acquisition of *tetK* gene and secondly by ribosomal protection which is achieved by acquisition of *tetM* gene which leads to competitive binding to the 30S subunit by a ribosomal protection protein (RPP) which interferes with the binding of drug.[15,16]

Binding to the 50S ribosomal subunit

Resistance to Chloramphenicol has been attributed to the acquisition of chloramphenicol acetyltransferase gene (*cat* gene) which inactivates chloramphenicol by acetylation and affects its binding to the 50S ribosomal subunit.[17] Resistance to other antimicrobials that affect the 50S ribosomal subunit is achieved by a common mechanism and is due to acquisition of the gene that code for the Erythromycin resistant methylases i.e. ermA, ermB, and ermC, these enzymes causes methylation of the 50S ribosome and bring about conformational changes that decreases the ability of these drugs to bind to ribosomes.[18-20] The oxazolidinones are newer drugs, and the only one currently in use is linezolid. Resistance to linezolid has found to happen via two mechanisms: mutation of the ribosomal RNA (rrn gene), and methylation of the ribosomal RNA (cfr gene). The cfr gene which encodes an RNA methyltransferase is plasmid-borne, and has also been shown to confer resistance to Chloramphenicol, Clindamycin, and Streptogramin drugs.[21,22]

3. *Affecting nucleic acid synthesis*

The fluoroquinolone drugs act by inhibiting gyrase or topoisomerase IV which halts DNA replication and transcription. Resistance to these drugs is a result of mutations in either the GyrA subunit of gyrase (encoded by gyrA gene), or the GrlA subunit of topoisomerase IV (encoded by grlA gene) which reduce the ability of the drugs to bind to their targets. In addition, some moderate resistance to norfloxacin (and possibly ciprofloxacin) is caused by induction of the NorA efflux pump (norA gene).[23]

4. Affecting metabolic pathway

The combination drug Sulfamethoxazole/Trimethoprim targets the folate biosynthesis pathway in bacteria by competitive inhibition. Resistance to Sulfamethoxazole is due to a mutation in the dhps (dihydropteroate synthase enzyme) gene which allows pABA to bind but has greatly reduced binding of Sulfamethoxazole. Resistance to trimethoprim occurs by a mutation in the dhfr (dihydrofolate reductase enzyme) gene with reduced binding of Trimethoprim.[23,24]

In our study we found that MRSA was the most common organism and is the most resistance to antibiotics amongst cases and controls. The antibiotics that have a 100% sensitivity are Vancomycin, Teicoplanin, Linezolid which shows that for the effective treatment of the

disease entity the patient has to be prescribed higher antibiotics which incur more cost to the treatment and also a risk of developing further resistance in instances where the compliance and affordability of the patient is poor, hence it should be recommended and guidelines should be formulated so proper dosage and duration of the treatment should be defined so that further antibiotic resistance should not progress which in turn will decrease the cost of treatment.

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