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Microbiology

Microbial profile of Chronic Suppurative Otitis Media with special reference to Pseudomonas aeruginosa in a Tertiary Care Hospital

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ABSTRACT Chronic suppurative otitis media is considered to be major problem in developing countries with relatively high mortality and morbidity (Glass cock et al; 1990). Pseudomonas aeruginosa is a significant pathogen among gram-negative organisms infecting ear (Brobby Gw et al ; 1987, Brook I et al., 1996). In recent year Pseudomonas resistant to multi drugs is of great concern to ENT surgeons.

- 110 Specimens were collected from 100 CSOM patients attending ENT op .
- 43 strains of Pseudomonas aeruginosa were isolated & Antibiotic susceptibility testing was done by Kirby - Bauer disc diffusion method.
- MIC detected using Hicomb test (E-TEST).

This study revealed 53.5% of pseudomonas aeruginosa isolates from CSOM is associated with multi drug resistance.

The challenge for physician and Surgeon to develop stricter pathogen surveillance and drug susceptibility testing and to follow Judicious utilisation of both older and newer antimicrobial agents.

KEYWORDS : Chronic suppurative otitis media, MIC, E test.

INTRODUCTION:

Ear is the most important sensory organ concerned with the perception of hearing. Infections are the leading causes of deafness worldwide. (CSOM Burden of illness and management WHO 2004). CSOM is a chronic suppurative inflammation of mucoperiosteal layer of the middle ear cleft. Incidence of CSOM higher in developing than developed countries particularly in India. The global burden of illness from CSOM involves 65-330 million individuals with draining ears, 60% of whom suffer from significant hearing impairment. CSOM accounts for a disease burden of over 2 million disability adjusted life (WHO 2004). It shows the importance of controlling the infections effectively by for preventing the hearing loss all over the world. Chronic suppurative otitis media is considered to be major problem in developing countries with relatively high mortality and morbidity (Glass cock et al; 1990).

Pseudomonas aeruginosa is an opportunist pathogen and infections are often severe and life threatening .They are difficult to treat because of the limited susceptibility to anti microbial agents. (Carmeli; Y; et al ; 1999).

The problem of antibiotic resistance in Pseudomonas aeruginosa is on the increase. Accumulation of resistance after exposure to various antibiotics and cross-resistant among agents may result in rnultidrug resistant Pseudomonas aeruginosa.So it is need of the hour to evaluate the multidrug resistant pseudomonas aeruginosa in chronic suppurative otitis media.

MATERIALS & METHODS:

All the Ear Swab specimens obtained from CSOM patient were processed in the Department of microbiology Government medical college hospital Coimbatore, for the period of 1 year.

SPECIMEN COLLECTION & PROCESSING:

The Bacteriological cultures were obtained from CSOM patients as per standard procedure. All the exudates and necrotic materials were removed from the external auditory canal using sterile cotton moist swab. Single use minitip culture swabs were used to harvest middle ear micro flora under vision and two Ear swabs were transported to the laboratory without delay. The status of the external auditory canal and tympanic membrane was inspected to determine the disease entities (ie otitis media). Two Swabs were collected from each patient and one was used to spread on clean glass slide and stained with Gram's Stain for immediate microscopic examination.

The specimens were processed as per standard protocol (Forbes et al, 1998; Sharma, 1988) for isolation of bacterial and fungal organisms. The following media were inoculated for isolation of the organisms; Blood agar, Chocolate agar, MacConkey agar, nutrient agar, cetrimide agar, brain heart infusion broth and Potato Dextrose Agar. All the plates and liquid media were incubated overnight at 37° C and observed for bacterial growth. Plates showing confluent growth in more than one media simultaneously were considered for the study. Pseudomonas aeruginosa colonies produced a yellow green pigment in cetrimide agar. The inoculated Potato Dextrose Agar was incubated at 27° C for fungal isolation.

Antibiotic susceptibility testing was done for these Pseudomonas strains by Kirby - Bauer disc diffusion method.

MIC DETERMINATION:

Antibiotics gradient strips (E -test) agar inoculation technique was used to determine the MIC. Himedia Hicomb MIC test strips were used in this study.The antibiotic gradient is created on the strip by applying different concentrations of antibiotics in repeated ways of an increasing number of small dots. When applied to the agar surface, the antibiotic diffuses into the surrounding medium in high to low amounts from one end of the strips to the other. The gradient remains stable after diffusion and the Zone of inhibition created takes the form of ellipse. The MIC is read only on the side of the comb at the point where the zone edge meets the strip edge.

ANTIMICROBIAL CONCENTRATION AND INOCULUM PREPARATION:

MIC for cefotaxime, Ceftazidime was determined by an antimicrobial gradient strips (E. test) agar inoculation technique on Muller - Hinton Agar. The gradient Concentration of Cefotaxime and ceftazidime is 240, 120, 60, 30, 15, 10, 7.5, 5, 3, 1, .1, .01, 0.001(mg/ml, The Test inoculam was prepared with an overnight growth of each isolate, which was adjusted to a turbidity equivalent to 0.5 McFarland standard. The test organism was inoculated in Muller - Hinton Agar plate. The inoculam was allowed to dry for 5 minutes with lid in place. The Hicomb MIC strip was applied on agar surface with the MIC scale facing downwards. Then plates were incubated at 37°C and examined after 24 hours. Pseudomonas aeruginosa ATCC 27853 was used as a reference strain in every batch of MIC tests

RESULTS:

In this study, a total of 110 Ear Swabs were collected from 100 Patients with CSOM attending ENT op Coimbatore Medical College hospital and Ear swabs were processed in the laboratory of microbiology department. Out of 100 patients with CSOM 10 patients were suffering from bilateral CSOM About 114 Organisms were isolated from 110 specimens. Among 110 specimens 105 numbers showed positive cultures (95.45%) This study showed that most common isolates obtained from CSOM patients were Pseudomonas aeruginosa (37.7%), followed by Staphylococcus aureus (32.4%), Proteus sp (12.2%), Klebsiella sp (7.8%), Coagulase negative Staphylococcus

aureus (2.6%), Streptococcus pyogens (1.7%), E. Coli (0.8%), Moraxella catarrhalis (0.8%), Candida Sp (2.6%), Aspergillus (0.8%). In total of 105 positive cultures 9 cultures showed mixed growth. The Organisms in the mixed growth were Pseudomonas aeruginoasa and Staphylococcus aureus in 3 cultures, Pseudomonas aeruginosa and Proteus in 2 cultures, Pseudomonas aeruginosa and Aspergillus in 1 culture, Staphylococcus aureus and Candida species in 3 cultures. Fungal growth was seen in 3.4 % of total isolation.

S.No	Name of the Organism	No. of isolates	Percentage
1.	Pseudomonas aeruginosa	47	37.7%
2.	Staphylococcus aureus	37	32.4%
3.	Proteus Sp	14	12.2%
4.	Klebsiella Sp	9	7.8%
5.	CoNS	3	2.6%
6.	Escherichia coli	1	0.8%
7.	Streptococcus pyogenes	2	1.7%
8.	Moraxella Catarrhalis	1	0.8%
9.	Candida Sp	3	2.6%
10.	Aspergillus Sp	1	0.8

Aerobic bacterial isolates in CSOM did not show any sex predilection though the females (53%) were more effected than male 47%. Common side of the CSOM infection was 52% Left side, 38% Right side and 10% in both side.

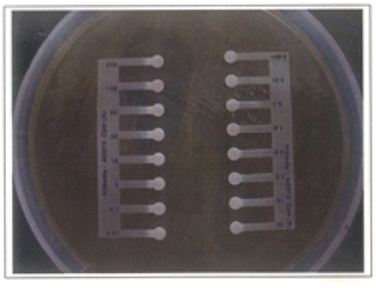
CSOM caused by pseudomonas aeruginosa were seen in all age groups with more cases in 21-40 years age group. Most of the patients affected were agricultural workers (34.8%) followed by construction workers 18.7% and school children18.7%.

The antibiogram showed the sensitive pattern as Gentamicin 46.5%, Amikacin 79.1% Tobramycin 46.5%, Ciprofloxacin 46.5%, ofloxacin 46.5%, Cefotaxime 46.5%, Ceftazidime 88.4%, Cefepime 86%, meropenam 93% and piperacillin 95.31%.

S. No	Name of the Antibiotics	No.of isolates Sensitive (%)	No. of isolates Resistance (%)
1	Gentamicin	20 (46.5)	23 (53.5)
2	Amikacin	3 (79.1)	9 (20.9)
3	Tobramycin	20 (46.5)	23 (53.5)
4	Ciprofloxacin	20 (46.5)	23 (53.5)
5	Ofloxacin	20 (46.5)	23 (53.5)
6	Cefotaxime	20 (46.5)	23 (53.5)
7	Ceftazidime	38 (88.4)	5 (11.6)
8	Cefepime	37 (86)	6(14)
9	Meropenam	40 (93)	3(7)
10	Piperaccillin	41 (95.34)	2 (4.66)

Pseudomonas aeruginosa grew in 43 cultures. The antibiogram pattern showed 23 isolates of pseudomonas aeruginosa (53.48%), were multi drug resistant (ie resistant to more than one antibiotic) Staphylococcus aureus (32.43%) Klebsiella Sp (33.37) and proteus species (14.2%).

MIC for cefotaxime and ceftazidime by Hi Comb (E. Test) method was found out for all the 43 strains of Pseudomonas aeruginosa. By this method 21 (48.8 %) strains were found to be resistant to cefotaxime and 5 (11.6%) strains were found to be resistant to ceftazidime.Strains showing MIC value > 16 mg/L were considered resistant for Cefotaxime and ceftazidime (includes intermediate and resistant categories) (Villanova 2000).



DISCUSSION:

Chronic suppurative otitis media is considered to be major health problem in the developing world with a relatively high morbidity and mortality. The overall prevalence of CSOM in these countries ranges from 5 -10 % (ManiJJ etal : 1987)). About 50% of brain abscesses are otogenic in origin with a mortality rate of 50 -75% (Sulla et al 1989).

A total of 110 Specimens collected and processed from chronic suppurative otitios media and 114 organisms were isolated. In which 9 patients had more than one organisms. Pseudomonas aeruginosa were isolated from 43 (37.7%) patients in our study which is similar to the studies by Berry S etal: 1996 &Samiullah et al: 2005. It was observed that pseudomonas aeruginosa has been isolated from all age groups but increased isolation was seen in the age group of 21-30 (41.8%).

In our study we noted multidrug resistance is more commonly seen in Pseudomonas aeruginosa 23 (53.48%) than Staphycococcus aereus 12 (32.4%), Klebsiella sp 3 (33.3%), proteus sp 2 (14.3%) The antibiogram of pseudomonas aeruginosa have showed sensitivity to Amikacin 79.1% , Ceftazidime 88.4%, Cefepime 86%, Meropenam 93%, Piperacillin 95.34% By considering Antibiogram first line of treatment for multidrug resistant is Amikacin or Ceftazidime or Cefepime and Second line is meropenam or piperacillin.

In the present study it was observed that 11.6% of strains were resistant to ceftazidime and 53.5% of strains were resistant to cefotaxime. Bouza observed a similar finding 15% strains were resistant to ceftazidime and Robert reported 46% of pseudomonas strains were resistant to cefotaxime. A major factor that has lead to the increase in antimicrobial resistant strains has been wide spread use of broad spectrum drugs including the use of third generation cephalosporins and fluoroquinolones.

Resistance to antimicrobial agents is an increasing public health threat. Multidrug resistance more prevalent among CSOM patients attending hospitals (or) community. Treatment with multiple antibiotic agents like cephalosporins and aminoglycosides specifically emerged as being important risk factors (Gould I.M. et al; 1994).

Conclusion:

CSOM lead among the causes of deafness and much importance to be given because of the morbidity they cause and also because of the increasing antibiotic resistance among bacterial isolates. Hence a composite study on the mechanisms of antibiotic resistance is mandatory to formulate antibiotic policies for controlling multi drug resistant infections.

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