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ORIGINAL RESEARCH

Evaluation of microbial pathogenicity in otitis media

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ABSTRACT
Otitis media is an inflammation of middle ear caused by bacterial pathogens and its response to antibiotics varied in different populations. The present study aimed to determine the prevalence of bacterial organisms and antibiotic sensitivity in otitis media. Samples were taken from 172 patients with otitis media visiting MAA ENT Hospitals, Hyderabad, India. Ear swabs were taken from the discharge/pus of ear and evaluation for the bacterial pathogens and their antibiotic sensitivity pattern was carried out by gram staining and culture sensitivity tests. Microbial etiology was detected in 66.3% of study cases, of which *Staphylococcus aureus* infection was the highest (37.2%) compared to *Pseudomonas aueroginosa* (24.4%), *Klebseilla pneumoniae* (3.5%) and *Cornybacterium diptheriae* (1.2%). Further, it was observed that the prevalence of *Staphylococcus aureus* was 58.3% in ASOM and 56.8% in CSOM while *Pseudomonas aueroginosa* is 33.3% in Acute suppurative otitis media and 37.7% in Chronic suppurative otitis media. *Staphylococcus aureus* (43.8%) was found to be more in subjects of >40 years while *Pseudomonas aueroginosa* (42.9%) and *Klebseilla pneumonia* (66.7%) affected 20-40 years of age group. *Staphylococcus aureus* was sensitive to azithromycin (71.9%) and amoxicillin (65.6%), *Pseudomonas aueroginosa* showed sensitivity to gentamycin (57.1%) and amikacin (54.8%), and *Klebseilla pneumoniae* to gentamycin (66.7%). *Staphylococcus aureus* and *Pseudomonas aueroginosa* contributed significantly for the occurrence of otitis media and the elderly age group was found to be highly affected. The antibiotic sensitivity of the study identified empirical antibiotics that can be used for treatment of otitis media and its associated complications by preventing the onset of resistant pathogens.

KEY WORDS: *Otitis media, Acute suppurative otitis media, Chronic suppurative otitis media, Staphylococcus aureus, Pseudomonas aueroginosa, Klebseilla pneumoniae.*

Introduction

Otitis media (OM) refers to an inflammation in middle ear cleft and is accompanied by effusion of fluids into the ear canal due to infection which may be associated with the presence or absence of tympanic membrane perforation (Pratt-Harrington, 2000). Nearly 90% of OM is mostly seen in younger children with less than 2 years of age but its occurrence may also be seen at adult age (Dhooge, 2003). The prevalence of OM varies in different countries, populations and ethnic groups (Lazo-Saenz *et al*., 2005).

The aetiology and duration of OM leads to a sequale of disorders such as acute suppurative otitis media (ASOM), chronic suppurative otitis media (CSOM) and otitis media with effusion (OME) (Browning, 2008). ASOM an acute form of OM, characterized by inflammation and the presence of fluid in the middle ear includes symptoms such as otalgia, irritability or fever (Qureishi *et al*., 2014). CSOM is a recurrent or persistent otorrohea over 2-3 weeks through a permanent tympanic membrane perforation leading to long standing inflammation of middle ear or serious complications such as hearing loss either temporarily or permanently (Qureishi *et al*., 2014). OME is a chronic inflammatory condition that is characterized by a nonpurulent effusion which may be either mucoid or serous (Van Zon *et al*., 2012).

OM is a multifactorial disease with an involvement of

aetiopathogenic factors including demographic, social, environmental, immunological, other health related factors like upper respiratory tract infections, allergy, asthma, eustachian tube dysfunction, cleft palate, and adenoid hypertrophy etc.,(Cripps *et al*.,2006; Adhikari *et al*., 2009). The presence of fluid in middle ear leads to microbial pathogenicity of OM which is usually associated with either bacterial or viral infections and is reported to range from 28% to 70% in the middle ear and nasopharynx (Yano *et al*., 2009; Vergison *et al*., 2010). Bacterial infections of the middle ear normally originate from the upper respiratory tract, with the bacteria entering the ear through the auditory (Eustachian) tube, the principal portal of entry to the ear. The most common bacteria involved in such infections are *Haemophilus influenzae*, *Streptococcus pneumoniaee, Streptococcus pyogenes, Staphylococcus aureus, Moraxella catarrhalis,* and *Pseudomonas aeruginosa* (Arol, 2005). Global reports show that *Hemophilus influenza* and *Streptococcus pneumoniae* are the most prevalent organisms responsible for otitis media (Yamanaka *et al*., 2008).

The usage of antibiotics in the treatment of OM have reduced the onset and reduced the progression of complications. Due to a wide range usage of antibiotics the bacterial pathogens have developed resistance and the isolates have become very common. The present study aimed to determine the prevalence of bacterial organisms within the subtypes of OM and their sensitivity to antibiotics in subjects with middle ear infections.

Subjects and Methods

Subjects

One hundred and seventy two patients with OM visiting MAA ENT Hospitals, Hyderabad, India, from 2011-2014 constituted the study subjects. Based on the otological examinations and clinical characteristics, OM was classified into ASOM and CSOM. The patient's age ranged from 2 to 72 years. The study was carried out by institutional ethics committee clearance and an informed consent was obtained from each patient to collect an ear discharge. Patient's ear was washed by normal saline (0.85% NaCl) and the discharge from the middle ear was obtained by the ENT specialist with the aid of an aural speculum before the instillation of any topical medication. Ear swab/discharge specimens from the middle ear which was aseptically collected were subjected to gram stain. The swabs were subjected to standard culturing techniques and the bacterial isolates were identified according to colony morphology and biochemical tests. After the identification of bacterial isolates their antibiotic sensitivity test was done using Kirby–Bauer method and the plates were read out after overnight incubation. By measuring the zone of inhibition around the antibiotic discs the activity of antibiotics on the bacterial isolates was determined.

Statistical analysis

The data obtained was coded for statistical evaluations. Appropriate statistical analysis was performed using the Statistical Package for Social Sciences, PASW STATISTICS 18.0 software (SPSS Inc., Chicago, IL, USA). Continuous data is represented as means whereas categorical data as proportions. The results were represented in tables and figures

Results

Among the study subjects, the prevalence of CSOM was 83.7% which was more compared to ASOM (16.3%). The patients mean age visiting the hospital was 36.4±18.71 years and the study subjects comprised 59% of males and 41% of females and with a male preponderance of 1.5. The occurrence of OM subtypes with respect to age is depicted in table 1.

The microbial pathogenicity was found to be positive in 66.3% the study subjects, of which *Staphylococcus aureus* (56.1%) was the most predominant bacterial pathogenic organism, followed by *Pseudomonas aeruginosa (*36.8%), *Klebseilla pneumoniae* (5.3%) *and Cornybacterium diptheriae* (1.8%). It was also observed that the prevalence of *Staphylococcus aureus* was 58.3% in ASOM and 55.6% in CSOM while the prevalence of *Pseudomonas aeruginosa* is 33.3% in ASOM and 37.8% in CSOM and *Klebseilla pneumoniae* is 8.3% in ASOM and 4.4% in CSOM (Table 1). The prevalence of *Staphylococcus aureus* was 43.8% in subjects >40 years while *Pseudomonas aeruginosa* was 42.9% and *Klebseilla pneumoniae* was 66.7% in the age

group of 20-40 years *(*Figure 1).

Table 2 and 3 revealed that *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the important pathogens of otitis media that have sensitivity and resistance to many antibiotics. In the present study, *Staphylococcus aureus* revealed a high level of sensitivity to amoxycillin (65.6%) and azithromycin (71.9%) and high level of resistance to vancomycin (75%) and gentamycin (65.6%). It was observed that *Staphylococcus aureus* showed more sensitivity to amoxicillin and azithromycin while resistant to vancomycin and cefatoxime in ASOM subjects. In case of CSOM, *Staphylococcus aureus* showed high resistant to vancomycin and gentamycin and more sensitivity towards amoxicillin and azithromycin (Table 2).

Most of the *Pseudomonas aeruginosa* isolates of OM were sensitive to amikacin (54.8%) and amoxicillin (57.1%) while resistant to linezolid (61.9%) and vancomyin (69%). In case of ASOM, amoxicillin (75%) showed more sensitivity to *Pseudomonas aeuroginosa* while resistant to amikacin (87.5%) and linezolid (75%). It was also reported that *Pseudomonas aeuroginosa* was highly sensitive to amikacin (64.7%) and gentamycin (58.8%) while resistant to vanocmycin (70.6%) and linezolid (58.8%) in CSOM (Table 3). In case of *Klebseilla pneumoniae* more sensitivity was noticed in gentamycin (66.7%) for both the forms of OM.

Parameters	ASOM (n=28)	CSOM (n=144)	Total (n=172)
Gender			
Male	18(64.3)	84(58.3)	102(59.3)
Female	10(35.7)	60(41.7)	70(40.7)
Age(years)			
0-20	2(7.1)	40(27.8)	42(24.4)
20-40	14(50.0)	46(31.9)	60(34.9)
>40	12(42.9)	58(40.3)	70(40.7)
Bacterial culture pathogens			
Positive			
Staphylococcus aureus	14(50.0)	50(34.7)	64(37.2)
Pseudomonas aeruginosa	8(28.6)	34(23.6)	42(24.4)
Klebseilla pneumoniae	2(7.1)	4(2.8)	6(3.5)
Cornybacterium diptheriae	Nil	2(1.4)	2(1.2)
Negative	4(14.3)	54(37.5)	58(33.7)

Table 1: Distribution of gender, age and bacterial pathogens in Otitis Media subtypes

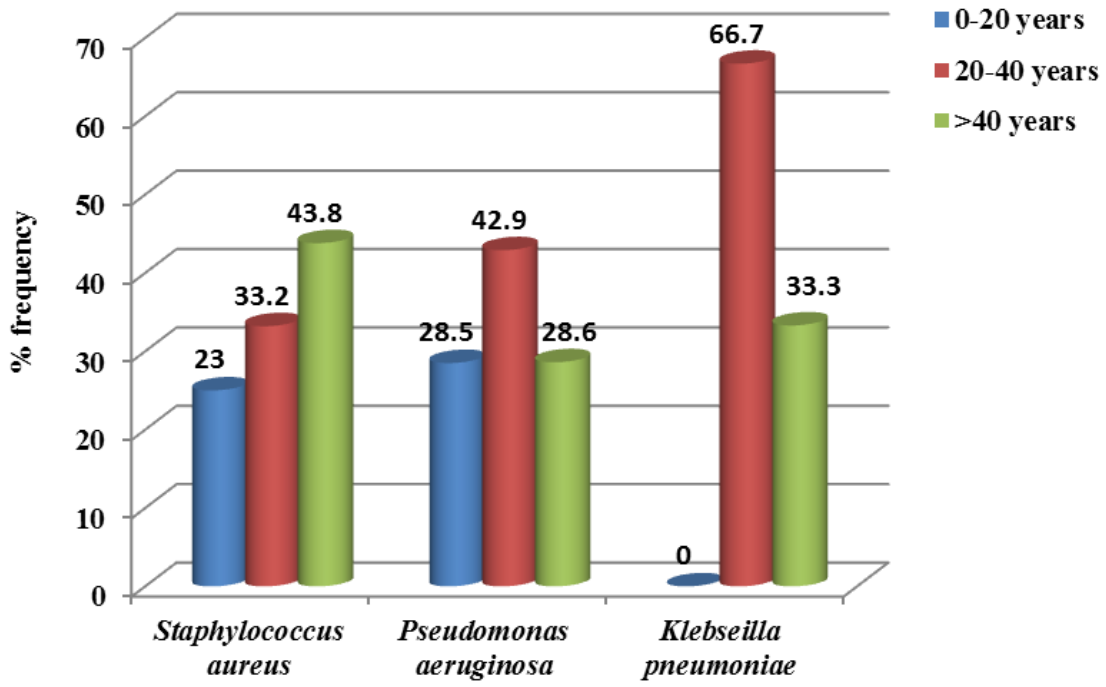

Figure 1: Prevalence of bacteria among Otitis Media subtypes in relation to age

Drugs	ASOM (n=14)		CSOM (n=50)		Total (n=64)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Amoxycillin	8(71.4)	6(28.6)	34(68)	16(32)	42(65.6)	22(34.4)
Azithromycin	9(64.3)	5(35.7)	37(74)	13(26)	46(71.9)	18(28.1)
Vancomycin	2(14.3)	12(85.7)	14(28)	36(72)	16(25)	48(75)
Gentamycin	6(42.9)	8(57.1)	16(32)	34(68)	22(34.4)	42(65.6)
Linezolid	6(42.9)	8(57.1)	19(38)	31(62)	25(39.1)	39(60.9)
Cefatoxime	5(35.7)	9(64.3)	21(42)	29(58)	26(40.6)	38(59.4)

Table 2: Antibiotic susceptibility pattern of *Staphylococcus aureus*

Drugs	ASOM (n=8)		CSOM (n=34)		Total (n=42)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Amikacin	1(12.5)	7(87.5)	22(64.7)	12(35.3)	23(54.8)	19(45.2)
Vancomycin	3(37.5)	5(62.5)	10(29.4)	24(70.6)	13(31)	29(69)
Gentamycin	4(50)	4(50)	20(58.8)	14(14.2)	24(57.1)	18(42.9)
Linezolid	2(25)	6(75)	14(41.2)	20(58.8)	16(38.1)	26(61.9)
Cefatoximne	3(37.5)	5(62.5)	15(44.1)	19(55.9)	18(42.9)	24(57.1)
Ciprofloxicin	4(50)	4(50)	18 (52.9)	16(47)	22(52.4)	20(47.6)

Table 3: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

Discussion

OM is one of the common inflammatory disorders of middle ear which has an important health concern at early life as well as in adults. The prevalence of OM varies widely and causes a serious burden of illness globally. However, studies on prevalence of OM are limited in Indian population. Most of the earlier reports indicated the prevalence of OM in younger age group but there is scarcity of data available in other age groups (Bernstein, 1992; Dhooge, 2003). The prevalence of CSOM was more in the younger age group which was similar to studies of Shyamala *et al*., 2012. The observations of present study revealed the occurrence of male preponderance in otitis media while some studies also reported female preponderance (Teele *et al*., 1989).

Microbial studies have reported that *Streptococcus pneumoniae, Hemophilus influenzae,* and/or *Moraxella catarrhalis* are the most common bacterial pathogens leading to ASOM (Chonmaitree, 2006). But the present study, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most prevalent bacterial pathogens in ASOM and CSOM when compared to *Klebseilla pneumoniae* varying with the other studies (Indudharan *et al*., 1999). The study of Indudharan *et al.,* 1999 have reported that *Pseudomonas aeruginosa* is the most common pathogen isolated around the world in CSOM and less commonly isolated is *Staphylococcus aureus*. The results of the present study on CSOM revealed that *Staphylococcus aureus* was the most commonly isolated pathogen followed by *Pseudomonas aeruginosa* which is in agreement with study done by Aslam *et al*., 2006. *Staphylococcus aureus* was the most prevalent form at an elderly age (>40 years) while *Pseudomonas aeruginosa* and *Klebseilla pneumoniae* in the middle age (20-40 years) group.

Most of the bacterial pathogens involved in the infection of OM have become more resistant to antibiotics. The study has shown that *Staphylococcus* species were highly resistant to vancomycin in 75% and gentamycin in 65.6% of the total study subjects while sensitivity with azithromycin was 71.9% and amoxicillin was 65.6% in our study. The results of sensitivity are similar in both the forms of OM while the pathogen was resistant to vancomycin and gentamycin in CSOM. All the OM subjects have shown sensitivity of *Pseudomonas aeruginosa* to amikacin and gentamycin. In case of ASOM, amoxicillin was found to show more antibiotic activity on *Pseudomonas*. Sensitivity of amikacin (64.7%) was more in CSOM which is in agreement with the study of Madana *et al*.,2011. It was also observed that *Pseudomonas aeruginosa* showed resistant to azithromycin and linezolid indicating the development of enzymes that prevent the action of antibiotics in the study population.

Bacterial pathology, especially the prevalence of *Staphylococcus aureus* and *Pseudomonas aueroginosa* was found to be significantly important microbes involved in the onset of OM. OM occurring at an elderly age group was found to be highly affected by the *Staphylococcus aureus* while *Pseudomonas aueroginosa* and *Klebseilla pneumoniae* bacterial infections is in middle age. Bacterial sensitivity revealed that *Staphylococcus aureus* in otitis media was more sensitive to amoxycillin and azithromycin antibiotics while gentamycin and amikacin showed more sensitivity on *Pseudomonas aueroginosa* and *Klebseilla*

pneumoniae to gentamycin. In conclusion, the present study of bacterial pathogens culture and sensitivity had provided suitable management strategies to be followed for OM and its associated complications

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