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Prevalence of Bacteria in Chronic Suppurative Otitis Media Patients and Their Sensitivity Patterns Against Various Antibiotics in Human Population of Gilgit

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Abstract.- The objective of this study was to determine the microbial profile and their antibiograms for Chronic Suppurative Otitis Media (CSOM) patients in Gilgit and the early effective antibiotic treatment based on the knowledge of causative agents. Discharge specimens from the ears of 57 CSOM patients were bacteriologically investigated by standard methods and the bacterial strains were identified using biochemical tests. The age of patients in this study ranged from under 1 year to 60 years with 18 patients under 10 years (39.2%) followed by 16 patients of 11-20 years (34.9%) and in the older age group of over 50 years, there was no infestation. Gender ratio amongst the 57 patients was 32 (56.1%) males and 25 (43.9%) females and infestation was also high in 25 males (43.9%) as compared to 21 females (45.6%). There were 46 (80%) patients with mono-microbial infestations the most common infestation was S*taphylococcus aureus* with 30 (65.2%) patients, followed by *Pseudomonas aeruginosa* 7 (15.2%), *Proteus mirabilis* 6 (13.0%) and *Escherichia coli* 3 (6.2%). Ceftriaxone was the drug of choice for treatment of CSOM, with an overall sensitivity amongst 41 (89.2%) patients and was followed by 38 patients sensitive to Ofloxacin (82.6%). Ampicillin and Cephalexin were the least effective drugs with sensitivities of 10 (2.17%) and 12 (26.1%) patients, respectively.

Key words: *Otitis media,* otitis media in Gilgit, middle ear infection, inflammation of middle ear.

INTRODUCTION

Chronic Suppurative Otitis Media (CSOM) is **a** prevalent infection worldwide (Osazuwa *et al*., 2011) and is the inflammation of the middle ear drum, inner ear and Eustachian tube (Arroll, 2005) caused by pathogenic-microorganisms that reside in the middle ear (Epko *et al*., 2009; Akinjogunla *et al*., 2011). The infection is solely dependent on the route by which infection reaches the middle ear and the main route by which this occurs is through the Eustachian tube (Daly, 1997; Akinjogunla and Eghafona, 2011).

According to the World Health Organization report 2004, the prevalence of chronic otitis media cases in the general population of South East Asia is approximately 5.2%. This is an important public health problem with substantial economic and societal cost (Report of WHO/CIBA 1996) and the USA alone, directly or indirectly, invests more than two billion dollars on CSOM (Marcy *et al*., 2001).

This disease is very common in children (Owen *et al*., 1993; Li *et al*., 2001) because the Eustachian tube is short and more horizontal in position as compared to the adult, moreover, children have a less developed immune system to confer sufficient resistance to bacteria (Weiner and Collison, 2003).

CSOM causes conductive and sensor neural hearing loss and adverse effects on child development (El-Sayed, 1998) that in some cases leads to the death of, annually, over 50,000 children worldwide (Rovers *et al*., 2006; Jane and Patil, 2012).

The chronic form of otitis media is a major problem in developing countries like Pakistan (Anwar-Us-Salam *et al*., 1997; Mansoor *et al*., 2009) and the risk of prevalence of the diseases become higher in people with low socioeconomic status. Poor living conditions, overcrowding, poor hygiene and malnutrition have been suggested as a basis for the widespread prevalence of CSOM (Alho and Rantakallio, 1990; Couzos *et al*., 2003; Mirza *et al*., 2008; Aich *et al*., 2009). The most common

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microorganisms found in CSOM are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Aspergillus spp* and *Candida spp*, but these organisms vary according to geographical areas (Anwar-Us-Salam *et al*., 1997; Ahmed *et al*., 2008).

In peripheral health centers, the culture and sensitivity facilities for discharge from ears of CSOM patients are not available and general practitioners treat the patients using their own preference of topical eardrops and systemic antibiotics. Selection of antibiotics is influenced by its efficacy, resistance of bacteria, safety, risk of toxicity and cost (Indudharn *et al*., 1999; Ahmed *et al*., 2008).

Knowledge of the pattern of local antibiogram is very important for efficient and cost effective treatment of otitis media patients. Literature survey has revealed that such studies have been conducted in most parts of Pakistan except Gilgit-Baltistan. Therefore, the main objective of this study was to address the shortfall and determine the microbial profiles, antibiograms of CSOM patients in Gilgit and compare studies conducted within Pakistan as well as the neighboring countries.

MATERIALS AND METHODS

CSOM patients attending the Clinic of Ear, Nose and Throat (ENT) specialist, who had no antibiotic treatment for the previous three days, were referred to the District Headquarters Hospital, Gilgit Laboratory for bacteriological investigation. The ear/ears discharge was collected with a sterile swab stick from each patient and the samples were aseptically cultured on MacConkey agar (Oxoid), Blood and Chocolate agars plates within three hours. The plates were incubated aerobically and Chocolate agar plates were incubated an-aerobically in a candle jar, moisturized with socked cotton at 37°C for 24-48 hours.

The colonies were identified by colony morphology and Gram stain. The Gram negative organisms were confirmed by setting biochemical test following the World Health Organization Manual for Laboratory Investigations of Acute Enteric Infections 1987 and the Gram positive bacteria by Gram stain, catalase and coagulase enzyme reaction tests.

The antibiotic sensitivity of the confirmed organisms was performed by disk diffusion method (Baurer *et al*., 1966; Ahmed *et al.*, 2008). One ml of each identified bacterial isolate was prepared from an overnight culture and adjusted to 0.5 McFarland Standard. A sterilized wooden swab was soaked in each culture and used to streak on Mueller-Hinton agar (MHA) plates and allowed to dry at room temperature. Commercially available sterile disks from Oxoid/Difco at specific concentrations of Ampicillin (30mg), Augmentin (30ug), Ofloxacin (5ug), Cephalexin (30ug) Cephradine (30ug), Ceftrixone (30ug), Cefotaxime (30ug) and Erythromycin (10ug) were placed aseptically on the pre streaked agar plates with sterilized forceps. *E. coli* ATCC 25922, sensitive to all these drugs, was used as a control and the sensitivity of the antibiotics was recorded by measuring the zone of inhibition around the discs for each of the isolated cultures in millimeters (mm).

The interpretation of the measurement for sensitive and resistant bacteria was made according to the manufacturer's standard zone size. Percentage resistance and sensitive zone sizes were calculated using the formulas PR=a/bx100 and PS=c/dx100.
Where PR is percentage resistance; a is number of resistant isolates; b is number of tested isolates; PS is percentage sensitivity; c is number of sensitive isolates and d is number of tested isolates against antibiotics.

Table I. Frequency of bacterial species distribution amongst Gilgit CSOM patients.

Name of infecting bacteria	No. of isolates	Percentage
Gram positive bacteria		
Staphylococcus aureus	30	65.2
Gram negative bacteria		
Pseudomonas aeruginosa	7	15.2
Proteus mirabilus	6	13.1
Escherichia coli	3	0.65
Total	46	100.00

RESULTS

During the whole study unilateral discharge was seen and Table I shows 57 specimens of CSOM

Table II.- Gender and age wise distribution of CSOM patients from Gilgit referred for bacteriological investigation of pathogenic bacteria.

Age group (Years)	Total patients investigated	Males	Females	Total infected and referred	Males	Females
0-10	23	13	10	18	12	06
11-20	20	12	08	16	08	08
21-30	07	03	04	06	03	03
31-40	03	02	01	04	02	02
41-50	02	00	02	02	00	02
51-60	02	02	00	00	00	00
Total	**57**	**32**	**25**	**46**	**25**	**21**

were investigated for bacterial infestation and 46 (80.70%) specimens were found to be infected with different bacteria. The highest number of cases were infected with *S. aureus* 30 (65.2%) followed by 7 *P. aeruginosa* (15.2%), 6 *Proteus mirabilis* (13.1%) and 3 *E. coli* (6.5%) patients**.**

Table II shows the age and gender wise distribution of the investigated patients and their infestation. Highest numbers of patients were investigated in age group less than 10 years followed by 11- 20 years and so on. In all the cases there was dominancy of males as compared to females. The infestation was also high or equal in all age groups of males except age group 41-50 years.

Table III shows the geographical distribution of suspected CSOM patients referred to the laboratory for bacterial investigation. Gilgit district had the highest number of patient referral of 48 (84.2%) and infestation was 42 (87.5%). From districts of Ghizer and Diamer, only 3 patients were referred and infestation was 1 (33.4%) in both districts. From District Astore, 3 patients were referred and infestation was 2 (66.7%).

Table IV shows that the highest CSOM infestation, 18 (39.2%) of *S. aureus*, *P. aeruginosa*, *Proteus mirabilis* and *E. coli* was found in age group< 10 years and was closely followed by 11-20 years age group at 16 (34.9%). In the age groups, 31- 40 years and 41-50 years group the infestations were 4 (8.7%) and 2 (4.9%), respectively, while in age group 51–60 years no infestation was observed.

Table V shows *in-vitro* antibiotic susceptibility profile of the bacteria, *S. aureus, P. aeruginosa*, *Proteus mirabilis* and *E. coli* isolated during the study in CSOM patients from Gilgit. The most sensitive drugs for all the bacteria was Ceftriaxone41 (89.2%) followed by Ofloxacin 38 (82. 6%), Cefotaxime 32 (69.6%), Cepheradine 29 (63.1%), Augmentin 28 (60.9%), Erythromycin 24 (52.2%) and the least sensitive drugs were Ampicillin 10 (2.9%), Cephalexin 12 (26.1%) and Cefixime 18 (39.2%).

Table III.- Geographical distribution of CSOM patients referred for bacteriological investigation of pathogenic bacteria in Gilgit.

Name of area	No. of specimens investigated	Total infestation and referral	Percentage infestation
District Gilgit	48	42	42 (87.5%)
District Ghizer	3	1	1 (33.4%)
District Diamer	3	1	1 (33.4%)
District Astor	3	2	2 (66.7%)
Grand total	57	46	46 (80.70%)

DISCUSSION

This study was conducted on 57suspected CSOM patients from all age groups and genders. There was an overall infestation of various bacteria of 80% and was high as compared to other studies conducted within Pakistan, 17 in Karachi, 20 in Bahawalpur and 26 in Korea and 27 in Istanbul, Turkey. The most common isolated pathogenic bacteria was *S.aureus,* followed by *P. aeruginosa*, *Proteus mirabilis* and the least isolated organism was *E. coli*. In Pakistan, a study conducted in Quetta also found *S. aureus* was the most commonly isolated organism (Ahmed *et al*., 2008). Similarly, high prevalence of *S. aureus* had been reported in a study conducted in Benin City, Edo State, Nigeria (Akingunla *et al*., 2011).

Table IV.- Age-wise distribution of bacterial spp. amongst patients with CSOM from Gilgit.

Bacterial spp. Isolated	Age group (Years)						Total
	< 10	11-20	21-30	31-40	41-50	51-60	
Staphylococcus aureus	11 (23.9)	2 (26)	03 (6.5)	03 (6.5)	01 (2.2)	00 (0.0)	30 (65.2)
Pseudomonas aeruginosa	04 (8.7)	00 (0)	01 (2.2)	01 (2.2)	01 (2.2)	00 (0.0)	07 (15.2)
Proteus mirabilis	02 (4.4)	02 (4.4)	02 (4.4)	00 (0.0)	00 (0.0)	00 (0.0)	06 (13.1)
Escherichia coli	01 (2.2	02 (4.4)	00 (0.0)	00 (0.0)	00 (0.0)	00 (0.0)	03 (6.5)
Total	**18 (39.2)**	**16 (34.9)**	**06 (13.7)**	**04 (8.7)**	**02 (4.9)**	**00 (00)**	**46 (100)**

In Korea, the prevalence of pathogenic bacteria was 54% amongst 2000 patients and the most common pathogenic bacteria in their study was also *S. aureus* and was followed by *P. aeruginosa* (Park *et al*., 2008). In Peshawar, a study conducted on 124 patients also isolated both *S. aureus* and *P. aeruginosa* (Arshad *et al*., 2004) and in studies from various parts of the world e.g. Kota Bharu (Malaysia), Singapore, Benin city (Nigeria), Bahawalpur, Karachi (Pakistan), found *P. aeruginosa* as the highest common organism (Indudharn *et al*., 1999; Lay *et al*., 2002; Osazuwa *et al*., 2011; Mirza *et al*., 2008; Mansoor *et al*., 2009). Both *S. aureus* and *P. aeruginosa* are the main etiological agents of CSOM patients.

The gender wise analysis of our study shows a variation in the ratio of suspected CSOM patients and their infestation. Our findings are supported by numerous other studies that males are at higher risk of CSOM than females (Ahmed *et al*., 1999; Li *et al*., 2005; Variya *et al*., 2002), but there are studies in which females have higher infestation (Akinjogunla and Eghafona, 2011).

In the age group data analysis, the highest numbers of suspected CSOM patients were from and 11-20 years and highest infestations were also found in this group. Our results are in agreement with other studies conducted both in Pakistan and foreign countries (Mansoor *et al*., 2009; Egbe *et al*., 2010; Akinjogunla *et al*., 2011; Osazuwa *et al*., 2011). This highest infection in < 10 years age groups could be due to physiological, anatomical and socio-cultural reasons (Li *et al*., 2005; Akinjogunla and Eghafona, 2011).

In Gilgit, the microbiological quality of the drinking water and sanitation practices are very poor (Ahmed *et al*., 2012; Ahmed *et al*., 2003) and both water and soil contributes greatly in the spread of diseases. CSOM is most probably caused by lack of personal hygiene due to lack of education with patients inserting objects/instruments contaminated with soil into their ears and or during swimming in dirty and stagnant water rivers (Li *et al*., 2005). Moreover, the CSOM microbes are environmental organisms (Anzai *et al*., 2008; Ceylan *et al*., 2008) and playing with and in soil outside the homes and schools can also transmit them to the ears.

In the area-wise investigations, the results revealed that a higher number of CSOM patients, *i.e.*, (44) were referred from the district of Gilgit as compared to other districts, like Ghizer, Diamer and Astor, where only 9 cases were referred. This high number of patients from Gilgit district as could be due to the availability of the hospital and laboratory facilities. The infestation of the pathogenic bacteria was also high in the patients referred from different villages of the Gilgit district as compared to other districts' villages.

The antibiotic sensitivity pattern of *E. coli* in this study reveals that only Ofloxine is the drug of choice to treat the CSOM patients and the isolated strains sensitivity is 66.6% with the other tested antibiotics such as Augmentin, Ampicilin, Ceftriaxone, Cefotaxime, Cephradin and Erythromycin. Additionally, the strains are 100% resistant to Cephradin and this is a very alarming situation as treatment of the patients in the peripheries with this antibiotic continues due to lack of sensitivity and testing facilities in our district.

The sensitivity pattern of *S. aureus* with commonly used antibiotics such as Ampicillin, Cefixime Cephalexin are worse *i.e.*, less than 50% and for Augmentin, Cefotaxime, Cephradin, Erythromycine and Ofloxacine are 70 to 90% and

Table V.- *In vitro* antibiotic susceptibility spectrum of bacterial species isolated from Gilgit CSOM patients.

Bacterial species	No. of isolates	Amp. No. (%)	Aug. No. (%)	Oflo. No. (%)	Ceph. No. (%)	Cephr. No. (%)	Ceftr. No. (%)	Ery. No. (%)	Cefi. No. (%)	Cefo. No. (%)
S. aureus	30	03 (10)	25 (83.0)	27 (90.0)	12 (40)	27 (90.0)	28 (93.0)	21 (70)	10 (33)	25 (83.0)
P. aeruginosa	07	03 (42.8)	01 (14.2)	04 (57.5)	00 (00)	00 (00)	05 (71.5)	01 (14.2)	03 (42.8)	02 (28.5)
P. mirabilis	06	02 (33.3)	00 (00)	04 (66.6)	00 (00)	00 (00)	06 (100)	00 (00)	03 (50.0)	03 (50.0)
E. coli	03	02 (66.6)	02 (66.6)	03 (100)	00 (00)	02 (66.6)	02 (66.6)	02 (66.6)	02 (66.6)	02 (66.6)
Total	**46**	**10 (2.17)**	**28 (60.9)**	**38 (82.6)**	**12 (26.1)**	**29 (63.1)**	**41 (89.2)**	**24 (52.2)**	**18 (39.2)**	**32 (69.6)**

Amp, Ampicillin; Aug, Augmentin; Oflo, Ofloxacin; Ceph, Cephalexin; Cephr, Cephradine; Ceftr, Ceftriaxone; Ery, Erythromycin; Cefi, Cefixime; Cefo, Cefotaxime.

for Ceftriaxone it is above 90%. The sensitivity pattern against *P. aeruginosa* and *Proteus mirabilis* are the worst for all the antibiotics used during this study.

CONCLUSIONS

Due to variation in climate, community, accessibility of medical care and prescription of antibiotics, the pattern of microbial flora and sensitivity pattern vary in CSOM patients. Therefore, it is very important to identify the causative agents of CSOM and their sensitivity pattern against various antibiotics before treatment of the disease in the patients.

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