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Original Article

Prevalence of Gram positive bacteria in the affected individuals of Otitis media with effusion from the indigenous population of Southern Punjab, Pakistan: first report

Prevalência de bactérias Gram-positivas em andivíduos afetados por Otite média com efusão da população indígena do sul de Punjab, Paquistão: primeiro relatório

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Abstract

Otitis media with effusion (OME) is a type of otitis media (OM) characterized by the presence of fluid behind intact tympanic membrane and is one of the most common diseases of early childhood. It is an infectious disease associated with the presence of many pathogenic bacteria in the middle ear of affected individuals. This study was aimed to determine the prevalence of Gram-positive bacteria from the middle ear of OME patients in the population of Southern Punjab, Pakistan. The incidence of OME under comprehensive healthcare setting was investigated in patients who consulted at the department of ear, throat and nose, Bahawal Victoria Hospital (BVH), Bahawalpur, from December, 2019 to May, 2021. Ear swabs were taken from affected and normal individuals. After culturing bacteria from the ear swabs, microscopic analysis and biochemical tests were performed to characterize the cultured Gram-positive bacteria. Out of 352 patients examined, 109 (30.9%) patients had OME. Age of the participants ranged from 14 to 50 years; individuals between the ages of 14 and 22 years had the highest infection rates, while individuals between 40 and 50 years had the lowest rate of infection. Tympanic membrane perforation, fever, cough, sore throat, ear pain and hearing problem showed association with symptoms of OME. Microscopic analysis and biochemical characterization showed the presence of streptococci and staphylococci in all the studied samples. The most frequently isolated bacteria were *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus with percentage* of 53.3%, 20% and 13.3% respectively. *Enterococcus faecalis* (6.6%) and *Staphylococcus epidermidis* (6.6%) were also identified in the studied samples. This study will help in the better medical administration of OME affected individuals.

Keywords: Otitis media with effusion, Gram-positive bacteria, Tympanic membrane, *Streptococcus pneumoniae* and Pakistan.

Resumo

A otite média com efusão (OME) é um tipo de otite média (OM) caracterizada pela presença de líquido atrás da membrana timpânica intacta e é uma das doenças mais comuns durante a primeira infância. Trata-se de uma doença infecciosa associada à presença de muitas bactérias patogênicas na orelha média dos indivíduos afetados. Este estudo teve como objetivo determinar a prevalência de bactérias Gram-positivas do ouvido médio de pacientes com OME na população do sul de Punjab, Paquistão. A incidência de OME em ambiente de saúde abrangente foi investigada em pacientes que consultaram no departamento de ouvido, garganta e nariz, Hospital Bahawal Victoria (BVH), Bahawalpur, de dezembro de 2019 a maio de 2021. Cotonetes de orelha foram coletados de indivíduos afetados e normais. Após a cultura das bactérias dos swabs auriculares, análises microscópicas e testes bioquímicos foram realizados para caracterizar as bactérias Gram-positivas cultivadas. Dos 352 pacientes examinados, 109 (30,9%) apresentavam OME. A idade dos participantes variou de 14 a 50 anos; indivíduos entre 14 e 22 anos apresentaram as maiores taxas de infecção, enquanto indivíduos entre 40 e 50 anos apresentaram as menores taxas de infecção. Perfuração da membrana timpânica, febre, tosse, dor de garganta, dor de ouvido e problema de audição apresentaram associação com sintomas de OME. A análise microscópica e a caracterização bioquímica mostraram a presença de estreptococos e estafilococos em todas as amostras estudadas. As bactérias isoladas com maior frequência foram *Streptococcus pneumoniae*, *Streptococcus pyogenes* e *Staphylococcus aureus* com percentuais de 53,3%, 20% e 13,3%, respectivamente. *Enterococcus faecalis* (6,6%) e *Staphylococcus epidermidis* (6,6%) também foram identificados nas amostras estudadas. Este estudo ajudará na melhor administração médica de indivíduos afetados por OME.

Palavras-chave: Otite média com efusão, bactérias Gram-positivas, membrana timpânica, *Streptococcus pneumoniae*, Paquistão.

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1. Introduction

Otitis media (OM) is a clinical disorder that causes conductive hearing loss and involves the presence of fluid behind the intact tympanic membrane of the middle ear without any signs of acute inflammation (Sadé et al., 1989). Children who experience hearing loss because of OM may experience problems related to speaking and reading (Cai and McPherson, 2017; Carroll and Breadmore, 2018; Khavarghazalani et al., 2016; le Clercq et al., 2017). OM can lead to a number of problems, including mastoiditis, cholesteatoma, meningitis, brain abscess, and lateral sinus thrombosis. OM is caused by a number of factors, such as environmental factors, viruses, bacterial load, age, allergies, upper respiratory tract infections (URTIs), lack of breastfeeding, smoking, a low standard of living, a large number of siblings, clinical history of family and daycare attendance (Brennan-Jones et al., 2015; Zhang et al., 2014). OM may also result from immune system flaws. The multigenic nature of the genetic susceptibility factors is known to have broad effects on host immunological responses. The most prevalent infectious agents, such as *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pyogenes, Staphylococcus aureus* and/or respiratory viruses, colonize and multiply in the nasopharynx. These pathogens travel from the nasopharynx to the Eustachian tube and infect the middle ear which may result in OM (Coticchia et al., 2013; Mofatteh et al., 2018). It has also been reported that *Streptococcus pneumoniae* causes OM, acute respiratory infections, pneumonia, meningitis and sepsis (Silva et al., 2022). Depending on the type and severity, OM is treated differently, ranging from antibiotics to surgery (Lieberthal et al., 2013).

OM is majorly divided into three categories: acute OM (AOM), recurrent AOM (rAOM), and OM with effusion (OME). OME is also known as oto-tubaritis, OM serosa or OM with chronic exudate (Paradise et al., 2002; Mudry and Young, 2020). Approximately 80% of the children may face this disease before the age of 4 years which makes OME as one of the most common diseases of early childhood. The most common symptom of the disease is mild hearing loss. This hearing loss is so mild that it remains undetected for a large period of time. This mild hearing loss can only be detected with the help of tympanometry devices (Rosenfeld et al., 2016). Otoscopy is commonly performed to diagnose the disease but it is not very sensitive as the large number of OME cases remain undetected (Xu et al., 2020). OME is a disease of middle ear and its health is directly related to the health of upper respiratory tract (URT) (Ngo et al., 2016). Many bacteria have been isolated from the middle ear of OME patients which include *Corynebacterium otitidis, Moraxella catarrhalis, Haemophilus influenzae, Streptococcus pneumoniae, Alloiococcus otitidis, Pseudomonas spp.* and *Staphylococcus spp.* (Ari et al., 2019; Baek et al., 2018; Boers et al., 2018; Chan et al., 2016, 2017; Enoksson et al., 2020; Jervis-Bardy et al., 2015; Johnston et al., 2019; Kolbe et al., 2019; Krueger et al., 2017; Liu et al., 2011; Val et al., 2018; Xu et al., 2020). Many of these bacteria are commonly present in healthy URT as well which is making difficult to associate the presence of these bacteria with OME (De Boeck et al., 2017; Frank et al., 2003; Stroman et al., 2001).

Current study was designed to study the prevalence of Gram-positive bacteria from the microbial flora of middle ear of OME patients who visited ear, nose, and throat (ENT) department of Bahawal Victoria Hospital (BVH) Bahawalpur, one of the biggest hospitals in the South Punjab region of Pakistan, from December 2019 to May 2021.

2. Materials and Methods

2.1. Enrollment of patients

We visited the ENT department of BVH from December 2019 to May 2021 to identify and enroll patients of OME from different areas of South Punjab, Pakistan. A detailed questionnaire was designed to record family history, age of onset, symptoms and the occurrence of OME. Endoscopy was performed to check for abnormalities in the internal structure of the middle ear. Written consent was obtained from the participants or their parents/legal guardians. The study was approved by the Institutional Bioethics Committee of the Islamia University of Bahawalpur, Pakistan.

2.2. Collection and culturing of Ear swab samples

Patients were enrolled for the study if they did not have any oral or systemic antibiotic treatment in the seven days prior to sample taken, had a tympanic perforation and ear drainage for more than two months. A fine, sterile cotton swab was used to obtain the middle ear discharge from the tympanic chamber. The ear was cleaned with normal saline before sample taking. The samples were delivered to the microbiology lab for additional identification procedures. Bacterial culturing was done according to the method described by (Kombade et al., 2021). Briefly, the method consists of inoculation of swabs on blood agar and MacConkey agar plates for 24-48 hours at 37°C for the optimal growth of bacteria.

2.3. Microscopic analysis

The simultaneous application of chemical reagents for a set amount of time was used to apply the Gram staining procedure. Crystal violet was used as the primary stain, followed by iodine as the mordant, ethanol or acetone as the decolorizer, and safranin as the counterstain. To distinguish between Gram-positive and Gram-negative isolates, stained slides were air-dried and examined under oil immersion (100X) through bright field microscope.

2.4. Biochemical identification of bacterial isolates

Identification of isolates was performed using Berge's Manual of determinative bacteriology. The dichotomous key was used to identify reported microbes causing OME.

We were interested in identification of Gram positive bacteria as there are many reports which showed the

prevalence of Gram positive bacteria such as, *S. pneumoniae, S. aureus and S. pyogenes is* higher as compared to Gram negative bacteria in the bacterial flora of middle ear of OME patients (Afolabi et al., 2012; Al-Alousi and Mayas, 2012; Agha and Al-Delaimi, 2021; Brook and Frazier, 1996; Rosenblut et al., 2001; Silva et al., 2021).

A catalase test was performed to differentiate between *Streptococcus* and *Staphylococcus*. The positive results were further confirmed through a coagulase test to find the occurrence of widely distributed *Staphylococcus aureus* with coagulase activity while the Novobiocin test was performed to confirm the occurrence of *Staphylococcus epidermidis* (Dawodu and Akanbi, 2021). The samples with negative results were cultured on Blood Agar plates to observe the three types of hemolysis: α- hemolysis (partial hemolysis), β-hemolysis (complete hemolysis) and γ-hemolysis (no hemolysis). The results were recorded after the validation of the Optochin Sensitivity test, Bacitracin test and NaCl (6.5%) based test (Burckhardt et al., 2017; Facklam et al., 1982).

3. Results

Three hundred and fifty two patients were examined by the physicians of the ENT department during the period of the study. The age of the patients ranged from 14 to 50 years. Figure 1 provides a breakdown of the incidence of OM in the various age groups.

Using the criteria (Figure 2), 109 (30.9%) out of 352 patients were identified as having OME; Out of total 109 selected individuals 58 (53.2%) males and 51 (46.8%) females had the symptom of ear discharge, which was the principal criterion for the selection. Some enrolled patients were belonging to same families and were close relatives of each other. Perforation of tympanic membrane was present as the second most commonly identified clinical problem with the incidence rate of 41.2% in the studied group along with the presence of fever with the same incidence rate (41.2%). Coughing, sore throat and hoarseness were the third most common problem with a percentage of 20.11%. The percentage of the studied individuals that had pain in their affected ear at least once from the presence of the disease was 23.7%. None of the studied individual had any speech problem whereas 6.4% of the totally studied individuals had some auditory problems.

3.1. Bacterial isolates cultured from ear swabs of OME patients

The distribution of bacterial isolates found in swab samples is displayed in Table 1 and Figure 3 while the results of biochemical identification are presented in Figure 4. Out of the total 45 bacterial isolates cultured from swab specimens, Gram-positive Cocci made up 100% of the population, out of these *streptococci* and *staphylococci* made up 80% and 20% of the population, respectively. 66.6% of the staphylococci were *Staphylococcus aureus*, whereas 33.3% were coagulase-negative staphylococci (*Staphylococcus epidermidis*). *Streptococcus pneumoniae* (66.6%), *Streptococcus pyogenes* (25%) and *Enterococcus faecalis* were the three most prevalent *streptococci* (8.3%) shown in Figure 3.

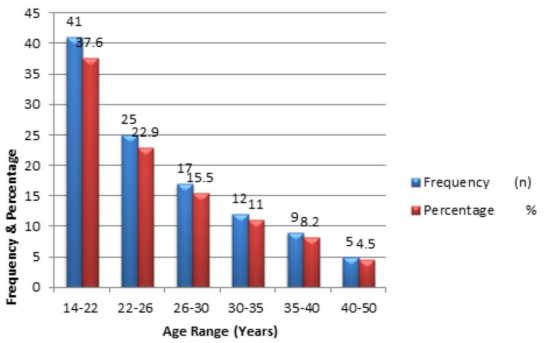

Figure 1. Prevalence of OME in different age groups.

4. Discussion

Middle ear irritation linked to OM is a prevalent condition in both children and adults (Korona-Glowniak et al., 2020). In order to identify the presence of Gram-positive bacteria in the bacterial flora of middle ear of OME patients and to link them with infection, 109 patients from Southern Punjab region of Pakistan were enrolled. We also investigated how common OM was within the analyzed age groups. The results showed that men (53.2%) had a higher prevalence of OM than did women (46.8%). These findings are in line with the findings of (Kombade et al., 2021), who found that while men engage in more outdoor

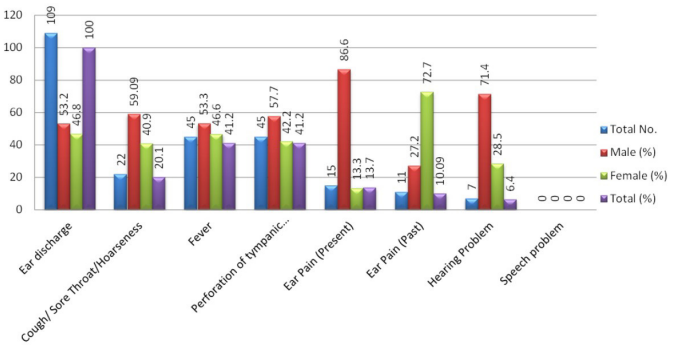

Figure 2. Clinical and diagnostic profile of subjects with OME.

Table 1. Profile of bacterial Isolates cultured from ear swabs of subjects.

Isolates	Total no. of isolates n=45	Total (%)	Gram Staining Gram +ive	Catalase test	Coagulase test	Blood Agar test	Optochin test	Bacitracin test	Growth in 6.5% NaCl	Novobiocin test
Enterococcus faecalis	3	6.6	Cocci (Single Diplo, chains Clusters)	-ive	_____	γ -hemolysis	_____	____	+ive	_____
Streptococcus pyogenes	9	20	Cocci (Single Diplo, chains Clusters)	-ive	_____	β-hemolysis	_____	+ive	_____	_____
Staphylococcus aureus	6	13.3	Cocci in grape-like clusters	+ive	+ive	____	_____	____	_____	_____
Streptococcus pneumoniae	24	53.3	Single cocci, lanceolate diplococci, Short chains of cocci	-ive	_____	α -hemolysis	+ive	____	_____	_____
Staphylococcus epidermidis	3	6.6	Cocci in grape like clusters	+ive	-ive	_____	_____	_____	_____	+ive

activities and travel more frequently than women (47.7%), they were more likely to get OM (52.2%) and recurrent URT infections. The frequency of OME infections is significantly influenced by age. In the current study, the age group with the highest infection rates was 14–22 (37.6%), followed by 22–26 (22.6%). In individuals from 40 to 50 years, a low distribution of OME (4.5%) was seen. Children's propensity for upper respiratory tract infections makes them obviously more vulnerable to OM. Studies show children are more susceptible to OM as compared to adults as children's immune systems are more sensitive and they have to deal with a bigger number of infectious bacteria. Poor hygiene, overcrowding, inadequate housing, low socioeconomic level, decreased immunity, and repeated upper respiratory tract infections are the major risk factors for increasing susceptibility of young children for OM (Kombade et al., 2021; Rathi et al., 2018). In our study individuals from the age of 14-22 years had the maximum percentage of affected individuals from the group of studied people. We did not find many children having OME who were fulfilling the set criteria. This may be because of many reasons including the unawareness regarding the disease as the region has one of the high percentages of illiteracy, poor socioeconomic background and difficulties to visit the hospital (Kumar and Seth, 2011; Mazlan et al., 2002).

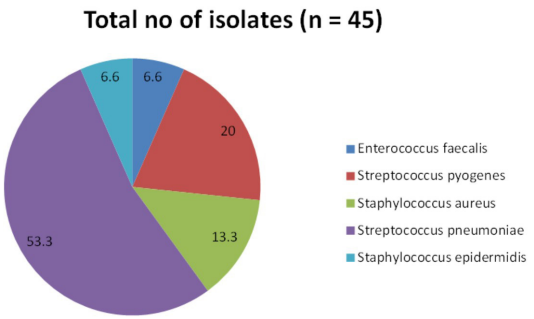

Figure 3. Percentage of bacterial strains isolated from cultured ear swabs from subjects.

Tympanic membrane perforation is one of the major clinical phenotype in the patients of OME. This perforation may be present in one ear or in both ears. In our study 45 (41.2%) affected individuals had tympanic membrane perforation in at least one ear. These results are in line with the studies conducted by (Kamal et al., 2004), (Ibekwe et al., 2009) and (Shrikrishna et al., 2013). The phenotype of hearing loss is also one of major finding in association with OME but we found hearing loss in a fraction of studied group (6.4%). Fever, cough and sore throat are the other parameters in which changes have been recorded in the patients of OME. We also checked these parameters and found the presence of these symptoms in 41.2% and 20.1% of the studied population, respectively. The presence of these symptoms indicates the role of ENT associated bacteria in OME and in associated problems. We used biochemical analysis to identify bacterial isolates in order to confirm the dispersion of infection-causing organisms. 100% of the bacteria identified for this investigation were Gram-positive, with *Streptococcus pneumoniae* being the most frequent isolate (53.3%). With 20% and 13.3% respectively, *Staphylococcus aureus* and *Streptococcus pyogenes* made up the majority. Similar findings were observed by (Silva et al., 2021), who observed *Streptococcus pneumoniae* (50.9%) as the most dominant bacterial pathogen in AOM patients, while the *Streptococcus pyogenes* (30.9%) was also widely distributed. According to many studies, *Staphylococcus aureus* is the most prevalent bacterium in people with chronic OM but in our study *Streptococcus pneumoniae* is the most commonly found bacteria. The different patterns of bacterial dissemination could be attributed to geographic variability (Afolabi et al., 2012; Al-Alousi and Mayas, 2012; Brook and Frazier,

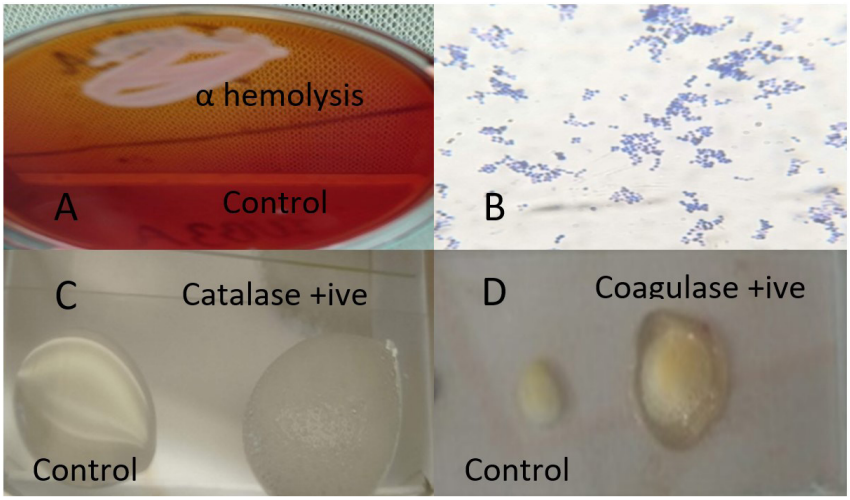

Figure 4. (A) Hemolysis on the blood agar media to differentiate the streptococci; (B) Microscopic observation of Gram positive *Streptococcus pneumoniae*; (C) Catalase test to differentiate the Streptococci and Staphylococci; (D) Coagulase test to differentiate the *Staphylococcus aureus* from *Staphylococcus epidermidis*.

1996; Rosenblut et al., 2001). Many studies showed the involvement of genetic factors in OME (Bartlett et al., 2015; Casselbrant et al., 1999; Crompton et al., 2017; Mira et al., 1999; Samuels et al., 2016; Santos-Cortez et al., 2016; Van Ingen et al., 2016). In our study, more than one individual affected with OME were present in some families. The presence of more than one affected individuals gives an indication about the association of genetic factors in these families and genetic screening can help to find out the involvement of any genetic factor in the enrolled patients.

5. Conclusion

We studied the population of Southern Punjab region of Pakistan having OME. This study indicates the OME to be associated with the presence of some or many symptoms liker tympanic membrane perforation, hearing problem, fever, cough and sore throat in certain individuals. We also identified the principal Gram-positive bacterial agents responsible for the illness in the Southern Punjab region of Pakistan. The most frequently isolated organisms were *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*. The findings of this study shed light on the Gram-positive harmful bacteria that may produce OM. Moreover, this is the first study, to the best of our knowledge, in which microbial flora of Gram-positive bacteria of the middle ear of OME patients from Southern Punjab region of Pakistan have been studied which will be helpful for the better management of the disease in the studied population.

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