Bacterial Aetiology of Pharyngotonsillitis in Paediatric Age Group in a Tertiary Care Hospital in Kerala

Thushara Ushakumari Bhuvanendran[1], Beena V.G.[2]

[1]Department of Microbiology, Bharath Hospital, Kottayam, Kerala, India.
[2]Department of Microbiology, Government Medical College, Kottayam, Kerala, India.

ABSTRACT

BACKGROUND

Pharyngotonsillitis is defined as a spectrum of conditions ranging from inflammation primarily confined to the tonsils to pharyngitis implying generalized inflammation of the whole of pharynx. Children are more prone to get several episodes of pharyngotonsillitis per year during their school years. Pharyngitis caused by *Streptococcus pyogenes* can cause two non-suppurative complications, acute rheumatic fever and acute glomerulonephritis which is responsible for significant morbidity and mortality. The present study was conducted to identify the prevalence of bacterial pathogens causing pharyngotonsillitis and to study their antibiotic sensitivity pattern that would indicate the optimum line of treatment.

METHOD

A total of 200 children at the age group of 2 - 12 years who had clinical features of pharyngotonsillitis according to the inclusion criteria were recruited for this study over a period of one year. With the help of a disposable wooden spatula, pus from the pharyngo tonsillar region was collected and processed. Predominant isolates obtained were identified and antibiotic sensitivity was done.

RESULTS

Bacteria was isolated from 70 samples. Pharyngotonsillitis was found most prevalent at the age group of 8 – 10 years. Intake of cold food stuffs and passive smoking at home was found to have statistically significant association as risk factor for pharyngotonsillitis. *Staphylococcus aureus* was the most common organism isolated followed by *Streptococcus pyogenes*. The other organisms isolated were group G and C streptococci, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* sub species (spp) aerogenes.

CONCLUSIONS

Staphylococcus aureus was the most common organism isolated, followed by *Streptococcus pyogenes*. All the isolates of beta haemolytic streptococci were found to be sensitive to penicillin. There was increased incidence of resistance to macrolides among the gram-positive isolates except *Streptococcus pneumoniae* and it may be due to the wide spread use of macrolides injudiciously. All the bacterial pharyngotonsillitis cases were cured with the antibiotic given according to the sensitivity except one case.

KEYWORDS

Pharyngotonsillitis, Acute Rheumatic Fever, Acute Glomerulonephritis, Bacterial Pathogens, Antibiogram

Corresponding Author:
Dr. Beena V. G.,
Associate Professor,
Department of Microbiology,
Government Medical College,
Kottayam, Kerala, India.
E-mail: drbeenasaji@yahoo.com

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BACKGROUND

Pharyngotonsillitis is defined as a spectrum of conditions, ranging from inflammation primarily confined to the tonsils to pharyngitis implying generalized inflammation of the whole of pharynx.[1] Viral pharyngitis account for 70 % of all pharyngitis, while bacteria cause only 20 – 40 % of the cases of pharyngitis. Group A streptococci is by far the most common bacterial cause of acute pharyngitis, accounting for approximately 15 – 30 % of cases in children and 5 – 10 % of cases in adults.[2]

Other streptococci which can also cause pharyngitis include group C and G streptococci. Other probable co-pathogens involved in causing pharyngitis include *Streptococcus pneumoniae*, *Cornynebacterium spp, Neisseria spp, Haemophilus spp, Staphylococcus aureus, Moraxella (Branhamella) catarrhalis, Treponema pallidum, Chlamydia pneumoniae, Arcanobacterium haemolyticum and Mycoplasma Pneumoniae.*[1,2] Gram negative bacteria isolated from pharyngitis are *Klebsiella spp, Pseudomonas aeruginosa* and *Proteus mirabilis*.[3] Viral causes of pharyngitis are adenovirus, rhinovirus, enterovirus, Ebstein Barr virus, influenza virus A and B, parainfluenza virus, respiratory syncytial virus, corona virus, herpes simplex virus (HSV) and cytomegalovirus.[4] Anaerobic bacteria involved in causing pharyngitis are Bacteroides sp, Fusobacterium sp, Peptostreptococcus sp and Veillonella sps.[5] Another cause of pharyngitis is candida which can cause oral thrush, and it is usually seen in immunocompromised patients.[1]

The potential non-suppurative complications of pharyngitis caused by group A streptococci are acute rheumatic fever and acute glomerulonephritis.[4] Among the post-streptococcal sequelae, acute rheumatic fever and rheumatic heart disease continue to occur in significant proportions in many parts of the world including India.[6,7] There is approximately 1 - 1.25 million cases of rheumatic fever or rheumatic heart disease in India. It is causing increased morbidity and mortality in patients. The high cost of surgical intervention for the treatment of latter complications of rheumatic fever or rheumatic heart disease put heavy economic burden on the families of the affected children. In order to prevent rheumatic fever or rheumatic heart disease in endemic communities, streptococcal pharyngitis should be diagnosed and treated without any delay.[7] Pharyngitis or tonsillitis is a common disease in children and usually a child will get one or two episodes of acute tonsillitis each year particularly in preschool or primary school age group.[8]

The suppurative complications of pharyngotonsillitis are peritonsillar abscess (quinsy), parapharyngeal space abscess, cervical lymphadenitis, sinusitis, otitis media and mastoiditis.[4] The present study was conducted to identify the prevalence of bacterial pathogens causing pharyngotonsillitis and to study their antibiotic sensitivity pattern that would indicate the optimum line of treatment. Thus, the timely treatment of pharyngotonsillitis can prevent non-suppurative complications like acute rheumatic fever, rheumatic heart disease and acute glomerulonephritis and suppurative complications.

Objectives

1. To identify and isolate the bacteria causing pharyngotonsillitis in patients attending ENT out-patient department (OPD) and paediatrics OPD in the age group of 2 - 12 years.
2. To find out the antibiotic sensitivity pattern of the bacterial pathogens isolated.

METHODS

A descriptive study was conducted at Department of Microbiology, Govt. Medical College, Kottayam in co-operation with the Department of ENT and Department of Paediatrics, Govt. Medical College, Kottayam for a period of one year from September 2015 to August 2016. For that, samples (throat swabs) were collected from 200 pharyngotonsillitis cases after getting consent.

Inclusion Criteria[3]

Patients with a history of

1. Sore throat,
2. Difficulty in swallowing,
3. Fever
4. And/or evidence of inflamed tonsils/pharynx. (Presence of erythema and exudates on the tonsils and/or pharynx on examination of the throat associated with cervical adenitis)

Exclusion Criteria

1. Patients who were receiving or had received antibiotics within two weeks of presentation.
2. Patients having peritonsillar abscess.
3. Children less than 2 years.
4. Patients who are not willing to give consent.

Throat examination was done to assess the presence of follicles, exudates, or membrane over the tonsils. The presence of lymph node enlargement, bad breath, palatal petechiae and adenoid hypertrophy were examined. Laboratory investigations results including anti-streptolysin O (ASO) titre were also collected.

After getting verbal consent of the patient with the help of a head lamp and a disposable wooden spatula which was used to depress the tongue, pus from the pharyngo tonsillar region was collected. The double swab was rubbed with rotation over one tonsillar area, then arch of the soft palate and uvula, the other arch of the soft palate, the other tonsillar area, and finally the posterior pharyngeal wall. One swab was used for gram stain and the other swab was used for aerobic bacterial culture. Throat swab was inoculated on sheep blood agar, MacConkey agar, chocolate agar, and tellurite blood agar media. *Staphylococcus aureus* was streaked perpendicular to the streak line for the detection of *Haemophilus influenzae* and incubated at 37^{o}C under 5 – 10 % CO_2. After 18 - 24 hours of aerobic incubation, pathogens were isolated, identified and antibiotic sensitivity testing of the isolates was done according to clinical and laboratory

standard institute (CLSI) guidelines. Identification of the bacterial pathogens were done based on staining, cultural and biochemical properties using standard laboratory procedures.[9]

Bacitracin sensitivity test was carried out for isolates of β haemolytic streptococci. Bacitracin sensitive and cotrimoxazole resistant isolates were identified as *Streptococcus pyogenes* and grouping of streptococci was done using SLIDEX strepto plus by latex agglutination method. It is a latex agglutination test for grouping of Lancefield group A, B, C, D, F and G. The group specific antigen is enzymatically extracted from the streptococcal cell wall. The antigen in the extract is identified using latex particles sensitized with group specific anti-streptococcal antibody. If antigen is present, it reacts with the latex particle suspension to form visible clumping. Antibiotic sensitivity testing of all pathogenic isolates was done on blood agar or Muller Hinton agar by Kirby Bauer and stokes disc diffusion method as per CLSI guidelines. All penicillin resistant isolates of *Staphylococcus aureus* were tested for beta lactamase production by iodometric method.[9]

Statistical Analysis

The data was entered into the Microsoft Office Excel 2010 and analysis was done using Statistical Package for Social Sciences (SPSS) version 20 and the results were compiled in tabular and graphical representation using Microsoft Word 2010 and Microsoft Excel 2010

RESULTS

As per the inclusion and exclusion criteria, throat swabs were collected from 200 children with pharyngotonsillitis and these throat swabs were processed and the results were obtained as follows.

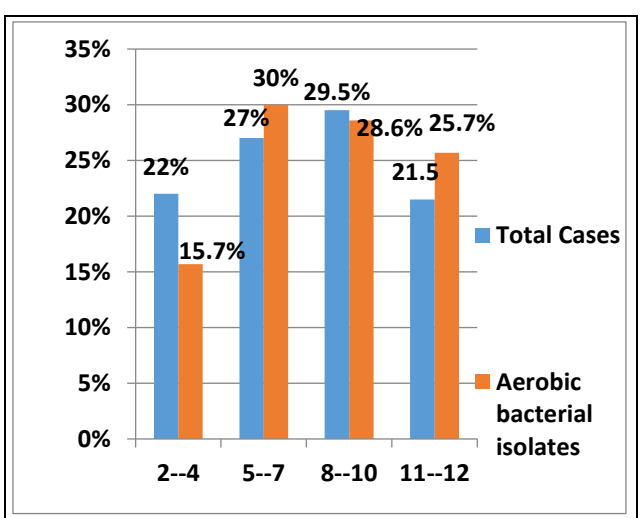

Figure 1. Age Wise Distribution of Study Group and Aerobic Bacterial Isolates

X axis – Age group, Y axis – Percentage of cases

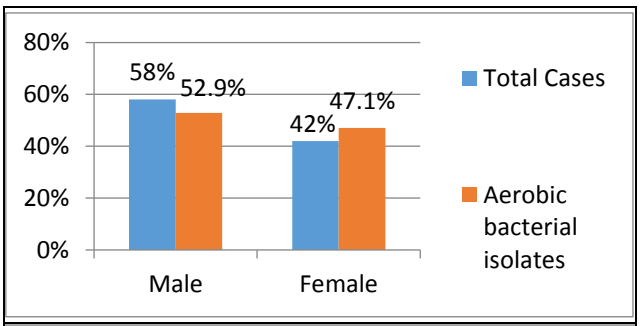

Figure 2. Gender Wise Distribution of Study Group

X axis – Gender, Y axis – Percentage of cases

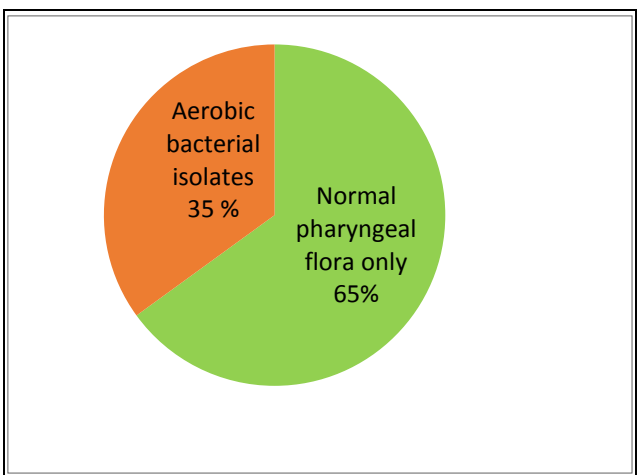

Figure 3. Culture Report of Study Population

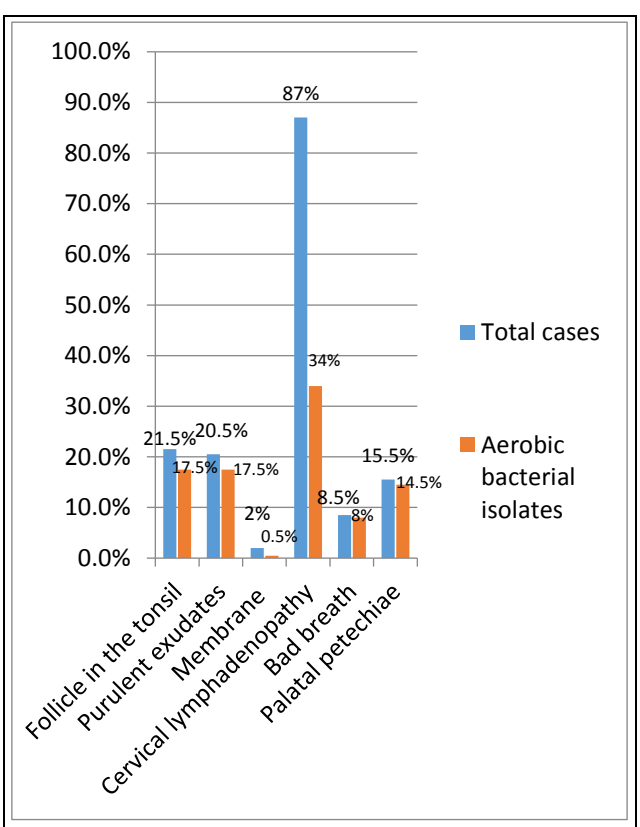

Figure 4. Distribution of Study Group Based on Signs

X axis – Signs seen in pharyngotonsillitis, Y axis – Percentage of cases

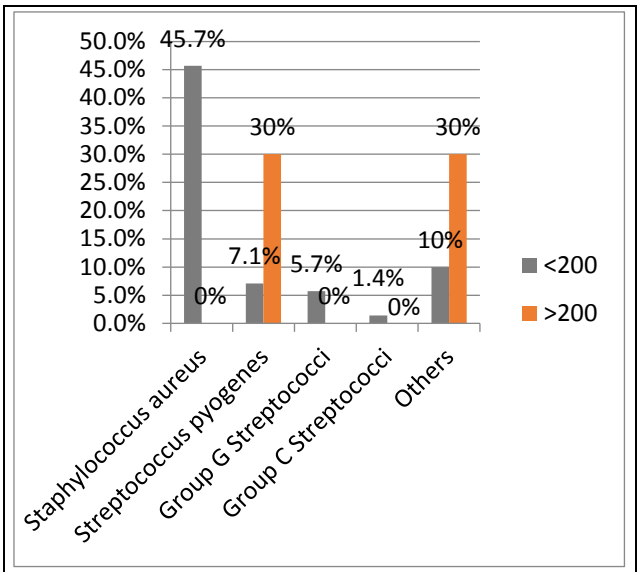

Figure 5. Association between ASO Titre and Pharyngotonsillitis

X axis – Bacterial isolates obtained in culture, Y axis – Percentage of cases

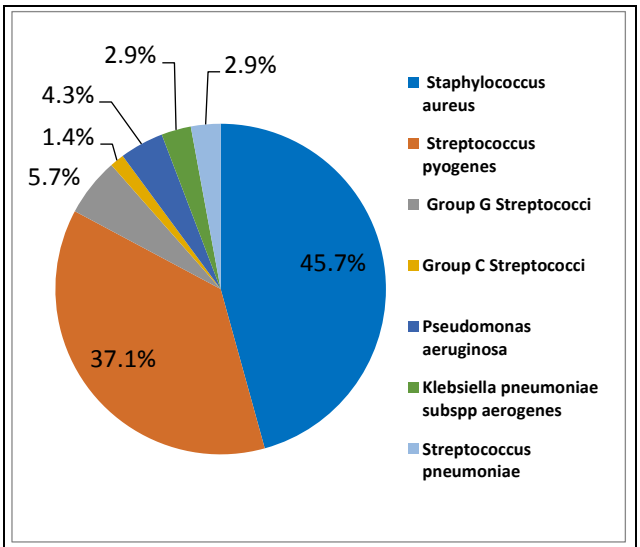

Figure 6. Percentage of the Isolates Obtained in Culture

DISCUSSION

The study was conducted to identify and isolate the bacterial pathogens and to determine their antibiogram in children with pharyngotonsillitis at the age group of 2 – 12 years in Government Medical College, Kottayam over a period of one year. Total of 200 cases were studied and 70 aerobic bacterial isolates were obtained.

In the present study, age group ranged from 2 to 12 years and it was noted that pharyngotonsillitis was most prevalent in the age group of 8 – 10 years (29.5 %) followed by 5 - 7 years (27 %), 2 - 4 years (22 %) and 1 – 12 years (21.5%). Similar result was obtained in a study done by Vijayashree et al. where maximum number of cases were obtained at the age group of 6 – 12 years.[10] This result is supporting the fact that pharyngotonsillitis is more commonly seen in preschool or primary school age group due to the spread of infection in class rooms.[11,12] Also the frequency of episodes of pharyngotonsillitis decreases with age which is due to increasing immunity. Considering the gender statistics, 58 % of the cases were male children and 42 % of the cases were female children. This result is in concordance with a study done by A. Agrawal et al. where 57.14 % of the cases were males and 42.86 % of the cases were females.[12] Of the 200 samples studied, from 70 samples bacterial isolates were obtained and rest of 130 samples yielded normal pharyngeal flora only. This result is similar to that of a study done by A.K Shrestha et al. where pathogenic bacteria were isolated from 36.6 % of the total pharyngitis cases.[13] Cough was seen more in culture negative cases than in culture positive cases which indicate a viral aetiology of those cases since cough, coryza, conjunctivitis, and diarrhoea are more common with viral pharyngitis. Of the total 200 cases, cervical lymphadenopathy was the most predominant sign (87 %) followed by follicles over the tonsil (21.5 %), purulent exudates over the tonsil (20.5 %), palatal petechiae (15.5 %), bad breath (8.5 %) and membrane over the tonsil (2 %). In a study done by M.S. Vijayashree et al. also cervical lymphadenopathy was the most predominant sign followed by follicles over the tonsil; and membrane over the tonsil was the least predominant sign.[14] Also cervical lymphadenopathy was present in majority of the culture positive cases.

Out of the 70 bacterial pharyngotonsillitis cases passive smoking was present in 32.9 % of cases and out of the non-bacterial pharyngotonsillitis cases, passive smoking was present in 33 % of cases. Chi square value and P value for the association of passive smoking as a risk factor for bacterial pharyngotonsillitis are 5.908 and .015 respectively. So, it is concluded that passive smoking at home was having statistically significant association as risk factor for bacterial pharyngotonsillitis. This finding is similar to that of the study done by Wilson E Sadoh et al.[3] ASO titre was above 200 Todd units in 21 cases in which *Streptococcus pyogenes* was isolated. Thus, out of the 26 cases in which *Streptococcus pyogenes* was isolated, there was rise in ASO titre in 21 cases. This finding is similar to the finding obtained in a study done by Rajesh K. et al.[14]

Out of the total 70 bacterial pharyngotonsillitis cases, 92.9 % were gram positive and only 7.1 % were gram negative. Predominant gram-positive bacterial isolate obtained was *Staphylococcus aureus* (45.7 %), followed by *Streptococcus pyogenes* (37.1 %), group G streptococci (5.7 %), *Streptococcus pneumoniae* (2.9 %) and group C streptococci (1.4 %). Gram negative isolates obtained were *Pseudomonas aeruginosa* (4.3 %), *Klebsiella pneumoniae* sub spp aerogenes (2.9 %). This finding is similar to the study done by Loganathan et al. where the predominant isolate was *Staphylococcus aureus* (40.9 %) followed by *Streptococcus pyogenes*, group G streptococci (6.1 %), *Pseudomonas aeruginosa* (3.8 %), *Streptococcus pneumoniae* (3.5 %), Klebsiella species (3 %), group C streptococci (3 %) and rest of the cases were *Haemophilus influenzae*.[15]

In another study done by Rajesh K et al. aerobic bacterial isolates obtained from surface specimen of tonsils were beta haemolytic streptococci (40 %), *Staphylococcus aureus* (21 %), Klebsiella spp (19 %), *Streptococcus pneumoniae* (12

%), proteus spp (5 %), and enterococcus species (2 %) and anaerobic bacterial isolates include bacteroides spp (7 %), fusobacterium spp (6 %) and peptostreptococcus spp (3 %). Gram negative isolates like *Pseudomonas aeruginosa* and Klebsiella spp were obtained in 4.3 % and 2.9 % respectively.[14] The two cases of *Pseudomonas aeruginosa* were suffering from AML (Acute myeloid leukemia) and they were undergoing chemotherapy. Immunosuppression due to chemotherapy may be a factor contributing to this infection.[16]

65.6 % of the isolates of *Staphylococcus aureus* were sensitive to penicillin and ampicillin in the present study. This pattern of sensitivity is similar to the study done by Agrawal et al. where 68.75 % of the isolates were sensitive to ampicillin[12] and Okoye E.L et al. where ampicillin is sensitive in 75 % of the cases.[17] In the present study, only 34.4 % of the isolates of *Staphylococcus aureus* were sensitive to macrolides erythromycin and azithromycin. In a study done by Babaiwa et al. only 9 % of the isolates were sensitive to erythromycin.[18] The increased resistance of *Staphylococcus aureus* to macrolides could be partly explained by the fact that macrolides were used as an alternative treatment of choice for infection caused by *Staphylococcus aureus* and *Streptococcus pyogenes* in patients with hypersensitivity to penicillin.[3] In the present study, all of the *Staphylococcus aureus* isolates were found to be beta-lactamase producers. In a study done by Kielmovitch et al. also, *Staphylococcus aureus* had the highest rate of beta-lactamase production on the tonsillar surface of children with recurrent tonsillitis.[19] The increased isolation of beta-lactamase producing bacteria in tonsillitis may be because of the effects of previous penicillin therapy.[20] Such organisms present in a localised soft tissue infection like tonsillitis could degrade penicillin in the area of infection, thereby protecting not only themselves but also penicillin susceptible pathogens like group A beta haemolytic streptococcal infections (GABHS).[16] The antibiotic sensitivity pattern shows that macrolides show a high level of resistance than penicillin and ampicillin.

In the present study, penicillin and ampicillin were sensitive in all the 26 isolates of *Streptococcus pyogenes*. This pattern of sensitivity is similar to a study done by Vanita Dhanda et al. where penicillin was sensitive in 99 % of the isolates and ampicillin in 92.2 % of the isolates.[21] Only 34.4 % of the isolates were sensitive to erythromycin and azithromycin. This result is similar to a study done by Shereen Mohamed Abd El Ghany et al. where 30 % of the isolates were sensitive to erythromycin.[22] All isolates of *Streptococcus pyogenes* were sensitive to first generation cephalosporin (Cephalexin). This is similar to a study done by Vanita Dhanda et al. where 100 % of the isolates were sensitive to *Streptococcus pyogenes*.[23]

Penicillin and ampicillin were sensitive in 100 % of the isolates of Group G streptococci. This is similar to a study done by Vanita Dhanda et al.[23] erythromycin and azithromycin are sensitive only in 25 % of the isolates. In the case of group G streptococci also the level of sensitivity to macrolides is less than penicillin and amoxicillin.

Only one isolate of group C streptococci is obtained which is sensitive to penicillin, ampicillin and cephalexin, intermediately sensitive to gentamicin and amikacin, and resistant to erythromycin and azithromycin. Sensitivity to penicillin and ampicillin are similar to the study done by Vanita Dhanda et al. where no isolates are resistant to penicillin or ampicillin.[23] 100 % of the isolates of *Streptococcus pneumoniae* were sensitive to penicillin and ampicillin, which is similar to the study done by M.S Vijayashree et al.[10] In the present study sensitivity to oxacillin was tested and 100 % of the isolates were found to be sensitive to oxacillin.

Gram negative isolates obtained in this study were found more resistant to the antibiotics tested than gram positive isolates. In the present study sensitivity of *Pseudomonas aeruginosa* to gentamicin and amikacin are 33.3 % which is similar to a study done by Agrawal et al.[12] 33.3 % of the isolates were sensitive to ciprofloxacin in the present study which is in contrast to the study done by Sevan H Bakir et al. where it is 100 % sensitive. 100 % of the isolates were resistant to ceftazidime which is in contrast to the study done by Sevan H Bakir where 9 % of the isolates are resistant to ceftazidime. Only 33.3 % of the isolates were sensitive to piperacillin-tazobactam, whereas 100 % of the isolates are sensitive to piperacillin-tazobactam in the study done by Sevan H Bakir et al. 100 % of the *Pseudomonas aeruginosa* isolates were sensitive to meropenem in the present study which is similar to the study done by Sevan H Bakir et al. where 91 % of the isolates were sensitive to meropenem.[16]

None of the isolates *Klebsiella pneumoniae* sub spp aerogenes were sensitive to ampicillin which is due to their intrinsic resistance. 100 % and 50 % of the isolates were resistant to gentamicin and amikacin in contrast to the study done by Wilson E Sadoh et al. where 16.7 % of the isolates were resistant to gentamicin.[3] 100 % of the isolates were sensitive to cephalexin and this sensitivity is similar to the study done by M.S Vijayashree et al. where 100 % of the isolates were sensitive to cephalexin. 100 % of the isolates were resistant to ciprofloxacin in contrast to the study done by Vijayashree et al. where only 20 % of the isolates are resistant to ciprofloxacin.[10] The antibiotic sensitivity pattern in the present study shows that the 2 strains of klebsiella species are comparatively more resistant to antibiotics than that isolated from other studies. Piperacillin-tazobactam and meropenem are not reported in the study since they are not used in treating tonsillitis.

In the present study, only one patient developed rheumatic fever and in rest of the 199 cases there was no other complication. The isolate obtained in the rheumatic fever patient was *Streptococcus pyogenes* and rheumatic fever was detected after one month. The child was not given full course of antibiotic treatment.

CONCLUSIONS

In the present study, 200 children with pharyngotonsillitis were studied for detecting the bacterial profile and antibiogram of the bacterial isolates. Most common age group affected was 8 - 10 years, followed by 5 - 7 years. Cervical lymphadenopathy was seen in majority of the cases. Follicles and purulent exudates over the tonsils were found

to be more in cases in which aerobic bacterial isolates were obtained. Passive smoking was found to have statistically significant association as risk factor for pharyngotonsillitis. Rise in ASO titre was seen in majority (80.7 %) of cases where *Streptococcus pyogenes* was isolated. 35 % of the total cases yielded aerobic bacterial isolates and the rest of the cases (65 %) yielded normal pharyngeal flora only. The most common bacteria isolated was *Staphylococcus aureus* (45.7 %) followed by *Streptococcus pyogenes* (37.1 %). The other isolates obtained were group G and C streptococci, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* sub spp aerogenes. Gram positive isolates were more sensitive to antibiotics than gram negative isolates. Among the gram-positive isolates except *Streptococcus pneumoniae*, increased incidence of drug resistance to macrolides is seen. *Streptococcus pyogenes* was found to be 100 % sensitive to penicillin and ampicillin. All the culture positive cases were cured with the antibiotic given according to the sensitivity except one case which developed rheumatic fever.

Treating patients according to the antibiotic sensitivity of the isolates could avoid surgical procedure like tonsillectomy in the future because of the controversial issues regarding the efficacy of this surgical procedure. So that it may be able to conserve an organ tonsil which form one of the first line of defence mechanism of the body against invading microorganisms, and also avoid surgical stress and complications of surgery. So, it is relevant to do a throat swab culture for patients suffering from pharyngotonsillitis.

Limitations of the Study

Limitations of the study was that viral detection tests like polymerase chain reaction (PCR) or enzyme linked immunosorbent assay (ELISA) were not done due to lack of feasibility. Anaerobic culture was not done, since throat swab is not an ideal sample for anaerobic culture.

My suggestion for future research is that various viral causes of pharyngotonsillitis can be studied using multiplex RT PCR tests.

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