Role of Bacterial Biofilm in Refractory Post Tympanostomy Tube Otorrhea

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ABSTRACT

Background/Aim: Bacterial biofilm formation has been implicated in the high rate of refractory otorrhea after tympanostomy tube (TT) insertion. The aim of the work was to assess the role of bacterial biofilm in refractory posttympanostomy tube otorrhea (PTTO) and to determine the types of bacteria that grow on the surface of the TT and their pattern of antibiotic susceptibility. Subjects & Methods: The study was carried out on 40 patients (22 males and 18 females) with TT insertion for treatment of secretory otitis media. Their ages were ranged from 5-13 years with mean of 9.2 ± 2.19 years. Patients are followed up with continuous ENT examination for about 12 months after the TT insertion and then patients were classified into two groups: group1(n=24): the patients not developed PTTO and group 2 (n=16): the patients developed PTTO. Patients of group 2 were treated with local ear drops of antibiotic/corticosteroid combination (ciprocorit). According to the results after treatment, group 2 was classified into two subgroups: group 2a (n=4): patients responded to treatment and group 2b (n=12): patients not responded to treatment. From each patient of group 2b, a swab was taken from the external canal and examined bacteriologically and the TT was removed and examined for detection of the bacterial biofilm by two methods (semiquantitative culture and Acridine Orange (AO) staining). The organisms isolated from tube culture were identified by the standard bacteriological methods, tested for antibiotic sensitivity and examined for slime (biofilm) production by two methods: Congo red agar (CRA) plate method and tube method. Results: There were no significant differences between the studied groups as regard age or sex distribution of the patients (P>0.05). Out of the 12 cases of PTTO, only 9 were positive by tube culture and S.aureus was the most frequently isolated organism (55.6%) followed by P.aeuroginosa (44.4%). 60% of the isolated S.aureus and 50% of the isolated P.aeuroginosa were multidrug resistant against most antibiotics used. When comparing AO staining with the semiquantitative culture, the AO staining had poor sensitivity and specificity (77.8 % and 66.7 % respectively). Only 8 out of 9 isolated strains were biofilm producer as detected by CRA method whereas only 7 were positive as detected by tube method. There was a very good agreement between the two methods.

INTRODUCTION

Otitis media with effusion (OME) is characterized by an accumulation of fluid in the middle ear behind an intact tympanic membrane, without the symptoms or signs of acute infection. This form of inflammatory middle ear disease is the most common reason for young children to visit their family doctor and to have surgery [1].

Failure of medical treatment of middle ear effusion frequently ends up with tympanostomy tube (TT) insertion. TT insertion, the placement of a small drainage tube in the tympanic membrane, is the most common surgical procedure performed in children under general anesthesia [2].

The most common postoperative complication associated with TT insertion is otorrhea, with wide range of incidence between 5 to 83% [3,4].

A few bacteriological studies support the hypothesis of tube otorrhea in younger children being a manifestation of acute otitis media (with airway-derived bacteria such as Streptococcus pneumoniae and Haemophilus influenzae in the discharge) that would require systemic antibiotics. In children older than 3 years of age, cultures mainly present bacteria from the skin; Staphylococcus aureus (S.aureus) and Pseudomonas aeruginosa (P.aeruginosa) [5]. Persistent post-tympanostomy tube otorrhea (PTTO) has been linked to microbial biofilms [6]. Biofilms are microbial communities of surface-attached cells embedded in a self-produced extracellular polymeric matrix. The organisms within this polymeric matrix, or glycocalyx slime layer, are relatively resistant to antibiotics and can become a source of persistent and relapsing infection, often necessitating the removal of the implanted material. The decreased susceptibility to
microbial agents within a biofilm arises from multiple factors, including physical impairment of diffusion of antimicrobial agents, reduced bacterial growth rates, and local alterations of the microenvironment that may impair activity of the antimicrobial agent. Furthermore, the proximity of cells within a biofilm can facilitate plasmid exchange and hence enhance the spread of antimicrobial resistance [7].

The aim of the current study was to assess the role of bacterial biofilm in refractory post tympanostomy tube otorrhea and to determine the types of bacteria that grow on the surface of the TT and to study their pattern of antibiotic susceptibility.

SUBJECTS, MATERIALS & METHODS

Study population & study design:
This work was carried out in Oto.Rhino.Laryngology and Medical Microbiology & Immunology Departments in Banha university during the period from August 2011 to August 2012. The study was carried out on 40 patients with TT insertion for treatment of OME. They were 22 males (55%) and 18 female (45%). Their ages were ranged from 5-13 years with mean of 9.2 ± 2.19 years. Patients are followed up for about 12 months after the TT tubes insertion with continuous ENT examination. Patients were classified into two groups according to the development of PTTO:
- Group 1 (n=24): the patients not developed PTTO.
- Group 2 (n=16): the patients developed PTTO.

Patients of group 2 were treated with local ear drops of antibiotic/corticosteroid combination (ciprocort). According to the results after treatment with ear drops group 2 was classified into two subgroups:
- Group 2a (n=4): patients responded to treatment with ear drops.
- Group 2b (n=12): patients not responded to treatment.

From each patient of group 2b, a swab was taken from the external canal and the TT was removed and examined for detection of the bacterial biofilm.

Samples:
1) Ear Discharge
The pinna was pulled outward and backward, after which the sterile swab stick was gently introduced into the external auditory meatus and then gently rotated and taken out. [8]

2) Tympanostomy tube
The TTs were removed using the crocodile forceps under complete aseptic conditions and collected in sterile containers. [8]

Methods:
A-For each T.T the following were done:
1- Maki's semiquantitative culture [9]; which consists of rolling the TT back and forth on surface of nutrient agar plate, incubated at 37ºC aerobically for 24 hrs, The TT was then placed back in a dry sterile tube and held for acridine orange staining.
2- Direct Acridine Orange (AO) staining [10], in which the TT was first fixed at 56°C for 2 minutes in a dry incubator, then immersed in AO staining solution for 3-5 minutes, rinsed with water, drained and air dried. Each TT was attached to a clean glass slide with adhesive tape and immediately examined under a fluorescence microscope.

B- Ear discharge culture: The ear swabs were immediately inoculated onto well-dried and labeled MacConkey agar, blood agar, chocolate agar and Sabouraud's agar. Gram staining was done by using standard Gram staining technique. The inoculated MacConkey, blood agar and Sabouraud's agar were incubated aerobically at 37°C for 18-24 hours while inoculated chocolate plate agar was incubated in carbon oxide jar at 37°C at 18-24 hours. Subculture was done after the first night incubation.

C-Bacterial identification: The isolated organisms (from TT and ear discharge culture) were identified by colony morphology, gram stained film and by biochemical reactions (BR). The BR of Gram +ve cocci include: catalase, coagulase, deoxyribonuclease (DNAse) and bacitracin sensitivity tests (Oxoid). The BR of Gram -ve bacilli include: sugar fermentation, indole, methyl red, voges prosekeur, citrate utilization, urease, H2S production, oxidase tests and hanging drop method to detect motility (Oxoid). Germ tube test was done to identify Candida albicans (Oxoid).

D- Antibiotic susceptibility test: was done for all isolates by a modified Kirby-Bauer disc diffusion method [11].

F. Detection of slime (biofilm) production by the isolated strains: It was done by two methods:
1) Congo red agar (CRA) plate method: [12]
The isolated strains were streaked onto the CRA and incubated overnight at 37°C and a further 24 hrs at room temperature. Slime producing strains appear as black colonies (+ve test), and slime non-producing strains appear red (-ve test).
2) Tube method: [13]

The plastic conical tubes (Falcon Plastics) were used to assay for adherence of slime producing strains. This tube method consists of inoculating 10 ml of trypticase soy broth with a loopful of organism and the cultured tube was incubated overnight at 37°C, then emptied of their contents, washed with phosphate buffered saline (PBS) (pH 7.3), dried and then stained with trypan blue. Excess stain was removed and tubes were washed. Slime production was judged to have occurred if a visible film lined the walls of the tube. Ring formation at the liquid-air interface was not indicative of slime production.

RESULTS

The demographic data of the studied groups are summarized in (Table 1). There were no significant differences between the studied groups as regard age or sex distribution of the patients (P>0.05).

In this study, we examined the ear discharges of patients of group 2b by bacteriological cultures and the results revealed that, 100% (n=12) of ear discharges were positive for culture. The distribution of the organisms are summarized in (Table 2).

Also, we examined the T.Ts of patients of group 2b by semiquantitative tube culture and by staining with AO stain. Out of the 12 cases of PTTO, only 9 were positive by tube culture while 8 were positive by AO staining and S.aureus was the most frequently isolated organism (n=5) (55.6%) followed by P.aeruginosa (n=4) (44.4%) (Table 2). AO staining method had poor sensitivity and specificity (77.8 % and 66.7 % respectively) in relation to tube culture method (Table 3).

Regarding antibiotic susceptibility of the organisms isolated from tube culture, 2 (50%) out of 4 P.aeruginosa strains (cases no. 13,22) showed multidrug resistance against most antibiotics used. Also, 3 (60%) out of 5 S. aureus strains (cases no. 7,12,15) were oxacillin resistant and exhibited resistance against most antibiotics used (Table 4).

In this study, we examined the slime production by S. aureus strains (n=5) and P.aeruginosa strains (n=4) by both CRA plate test and by tube method and the results showed that 4 (80%) out of 5 S. aureus strains were biofilm producer as detected by CRA method whereas only 3 were positive as detected by tube method (80% strength of agreement between the two methods) (Table 5) while all (100%) P.aeruginosa strains (n=4) were biofilm producer as detected by CRA method as well as by tube method (100% strength of agreement between the two methods) (Table 6).

Table (1): Demographic data of the studied groups.

<table>
<thead>
<tr>
<th>Gender (no.,%)</th>
<th>Group 1 (n=24)</th>
<th>Group 2 (n=16)</th>
<th>P value</th>
<th>Group 2a (n=4)</th>
<th>Group 2b (n=12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>14 (58.3%)</td>
<td>8 (50%)</td>
<td>0.6</td>
<td>2 (50%)</td>
<td>6 (50%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Females</td>
<td>10 (41.7%)</td>
<td>8 (50%)</td>
<td>0.75</td>
<td>2 (50%)</td>
<td>6 (50%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Age (Mean ± SD)</td>
<td>9.29±2.19</td>
<td>9.06±2.26</td>
<td>0.75</td>
<td>10.25±1.7</td>
<td>8.66±2.3</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Table (2): Microorganisms isolated from ear discharge and semiquantitative tube culture

<table>
<thead>
<tr>
<th>Case number</th>
<th>Microorganisms isolated from ear discharge</th>
<th>Microorganisms isolated from semiquantitative tube culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>S. aureus</td>
<td>S. aureus</td>
</tr>
<tr>
<td>8</td>
<td>P. aeruginosa</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>9</td>
<td>S. aureus</td>
<td>S. aureus</td>
</tr>
<tr>
<td>12</td>
<td>Mixed infection (S. aureus &amp; Diphtheroides &amp; Candida albicans)</td>
<td>S. aureus</td>
</tr>
<tr>
<td>13</td>
<td>P. aeruginosa</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>15</td>
<td>S. aureus</td>
<td>S. aureus</td>
</tr>
<tr>
<td>18</td>
<td>Candida albicans</td>
<td>-ve</td>
</tr>
<tr>
<td>22</td>
<td>P. aeruginosa</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>27</td>
<td>Mixed infection (S. aureus &amp;S. epidermidis &amp; Candida albicans)</td>
<td>-ve</td>
</tr>
<tr>
<td>31</td>
<td>P. aeruginosa</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>33</td>
<td>S. aureus</td>
<td>S. aureus</td>
</tr>
<tr>
<td>38</td>
<td>S. aureus</td>
<td>-ve</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>9</td>
</tr>
</tbody>
</table>
Table (3): Relationship between results obtained by direct tube staining with AO stain and by the semiquantitative tube culture

<table>
<thead>
<tr>
<th>AO stain</th>
<th>Tube culture + ve</th>
<th>-ve</th>
<th>Total</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ ve</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ve</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>77.8*</td>
<td>66.7*</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>3</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The sensitivity and specificity of AO staining of TT in relation to the semiquantitative tube culture.

Table (4): Antibiotic susceptibility pattern of the organisms isolated from tube culture

<table>
<thead>
<tr>
<th></th>
<th>S. aureus (n=5)</th>
<th>P. aeruginosa (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case 9</td>
<td>Case 12</td>
</tr>
<tr>
<td>P</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>OX</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>VAN</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>CIP</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>AZM</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>CN</td>
<td>R</td>
<td>S</td>
</tr>
</tbody>
</table>

P, Penicillin; OX, Oxacillin; VA, Vancomycin; CIP, Ciprofloxacin; AZM, Azithromycin; CN, Gentamycin; AK, Amikacin; CAZ, Ceftazidime; MEM, Meropenem; PRL, Piperacillin; R, Resistant; S, Sensitive

Table (5): Results of slime production by S. aureus by tube method and by Congo agar method

<table>
<thead>
<tr>
<th>Tube method</th>
<th>Congo red agar</th>
<th>Kappa value</th>
<th>The strength of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>-ve</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Table (6): Results of slime production by P. aeruginosa by tube method and by Congo agar method

<table>
<thead>
<tr>
<th>Tube method</th>
<th>Congo red agar</th>
<th>Kappa value</th>
<th>The strength of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>-ve</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

DISCUSSION

PTTO remains the most common complication of tube placement, and studies show that its incidence ranged from 5 to 83% [3,4]. This wide range of the incidence of PTTO makes us think to study the PTTO and its incidence in our practices.

Microbial biofilm has been linked to persistent PTTO and TT obstruction [6]. The role of biofilm in PTTO has been a matter of debate. TT biofilm formation has been implicated in the development of PTTO occurring relatively late after TT placement. TT surface treatment aimed at reducing microbial adherence and biofilm formation has reduced early PTTO suggesting that biofilm formation has been implicated as a cause of both early and late PTTO [14].

A number of studies have looked at the use of silicon-coated and silver oxide impregnated tubes as a possible means of reducing the PTTO. In a study on 100 children who received either a surface treated silver oxide tube or a silver-oxide-impregnated tube in one ear while the other untreated ear used as a control it was found that these special tubes had no effect on the incidence of PTTO [15]. Another study was done to compare the post-operative complication rates of phosphorylcholine-coated fluoroplastic TT versus uncoated fluoroplastic TT and the authors concluded that phosphorylcholine-coated fluoroplastic...
ventilation tubes do not offer any advantages over uncoated standard fluoroplastic TT [16].

According to these results, we used in our study uncoated standard fluoroplastic TT that it cost effective to and gives the same results of phosphorylcholine-coated fluoroplastic tubes and the surface treated silver oxide tubes.

This study was carried on 40 patients, only 16 patients (40%) developed PTTO. These results agreed with that by Coat [4] who found that the incidence of PTTO ranges from 5% to 38%. In contrast, our results were higher than that of Gross et al. [17] who reported that the incidence of PTTO between 10% and 29%, however, were much lower than that of Charnock [18] who found that the incidence of PTTO is 74% and that of Ah-tye et al. [3] who reported that the incidence of PTTO is up to 83%.

Backer and Chole [19] stated that there was a significantly lower rate of the PTTO among treated patients with prophylactic local ear drops (0% during the first 2 weeks) than among the untreated patients that may explain the wide range of incidence of the PTTO that affected by the use of the prophylactic post-operative ear drops. The incidence of the PTTO is also affected with the timing of the TT placement, that patients having the TTs during the summer seasons are more liable to PTTO due to the water contamination. The incidence of PTTO is also higher in the children who had mucopurulant discharge at the time of surgery and children who had a recurrent upper respiratory tract infection or incipient otitis media.

There are several methods available for treating tube infections with otitis media. Topical treatment with ear drops is widely used irrespective of the indication for tube insertion. Many clinicians interpret the condition as acute otitis media and prescribe systemic antibiotics. Others prefer a wait-and-see policy claiming that the natural course is so benign that no treatment is required [20].

In a study done by Granath et al. [20], fifty patients were allocated to either of two treatment groups and were monitored for 6 months. Group I received only topical treatment (commercially available ear drops and saline solution). Group II was treated with topical treatment together with systemic antibiotics. All episodes of acute otitis media were registered. Bacterial samples from the ear discharge were taken. It was found that topical treatment alone might be used as first treatment of choice and the study do not support use of systemic antibiotics for tube associated otorrhea in children in general.

In 2001, Morpeth et al. [21] compared topical ciprofloxacin /hydrocortisone drops with neomycin /polymyxin b/hydrocortisone drops. With both agents, there was less otorrhea in the treated ears than in the untreated ears. However, because of ciprofloxacin lack of toxicity, Morpeth et al. [21] recommended it as the treatment of choice.

According to these finding, we used in our study ciprofloxacin /hydrocortisone local ear drops for the treatment of PTTO as there is no difference between the treatment with local ear drops alone or treatment with both local drops and systemic antibiotics.

Several papers have demonstrated biofilm formation on TT in vitro [22,23]. Several other reports indirectly indicate such formation in vivo by showing that differences in PTTO vary with tube materials [15,24]. One study has directly shown biofilms on TT in vivo in an animal model [25]. Another study done by Jang et al. [26] they evaluated biofilm formation on the surface of TT children with ciprofloxacin-resistant P. aeruginosa using a scanning electron microscopy and all specimens showed biofilm formation.

Our study is one of a few studies that evaluated biofilm formation on the surface of TT in vivo, we depend on follow up of patients with TTs for about one year, and the results revealed that 40% (16 out of 40) of patients developed PTTO, when this group of patients received local treatment, 75% (12 out of 16) of them not responded to treatment, and thus from each patient of this group, ear discharge sample was taken and examined bacteriologically and the TT removed and examined for biofilm formation. Ear discharge culture was positive in all (n=12) cases of PTTO, however only 9 out of 12 cases were positive by semiquantitative tube culture and 3 were negative. The negative tube culture in these 3 cases may be explained by that the organisms that cause PTTO in these cases may be non-biofilm producer as AO staining were also negative in these cases. Other explanation is that the samples were taken before these organisms not yet form a biofilm. All the organisms resulted from ear discharge culture were concomitant with those resulted from tube culture except in one case in which ear discharge culture yielded mixed organisms (S.aureus & Diphtheroides & C. albicans) while tube culture yielded only S.aureus. This may be explained by that Diphtheroides & C. albicans are part of the normal flora of the external canal.
In our study the results of the TT culture showed that *S. aureus* is the commonest organism that causes the PTTO with incidence of (55.6%) followed by *P. aeruginosa* (44.4%). These results are in agreement with that by Roland[27] who found that the bacteria of the skin (*S. aureus* and *P. aeruginosa*) are the commonest cause of PTTO in the children older than 3 years of age. Also, Brook et al.[27] stated that the PTTO in children older than 3 years caused by organisms that often arise from water exposure including *S. aureus*, *P. aeruginosa* and *S. epidermidis*, although there was no evidence of *S. epidermidis* infection in TT cultures in our study. Malaty et al.[28] stated that *P. aeruginosa* biofilm formation on TTs is enhanced by exposure to human blood, both while wet and after drying. Similar results have been found with other pathogens. Binding of *S. aureus* to biomaterials has long been known to be enhanced by the presence of the plasma protein fibronectin[29]. These results agreed with our results as we found high incidence of *S. aureus* & *P. aeruginosa* and this high incidence may be due to the blood exposure of the TT during the myringotomy incision.

When comparing AO staining with the semiquantitative tube culture, the AO staining had poor sensitivity and specificity (77.8 % and 66.7 % respectively). These results are near to that obtained by Coutlee et al.[30] where AO showed 71 % sensitivity and 77 % specificity.

In this study, two methods were used for detection of biofilm production by the isolated strains, the tube method and the CRA method. It was very good agreement between the two methods (80% in case of *S. aureus* and 100% in case of *P. aeruginosa*). This coincides with the results obtained by Freeman et al.[31] where there was very good agreement between both methods in 107 cases. Woznicova et al.[32] compared the CRA method with the tube method and they reported that the sensitivity and specificity of the former was (85% and 99%, respectively). So they stated that the CRA method for the detection of slime production seems to be a reliable tool. Thus the CRA is rapid, sensitive, specific and has the advantage that the colonies remain viable on the medium.

In this work, we studied the antibiotic susceptibility of *S. aureus* and *P. aeruginosa* strains isolated from tube culture and we found that most of the studied strains exhibited resistance to most antibiotics used. These results are in agreement with that by Astha and Amita[33] who found that antibiotic resistance among invasive, colonizing and commensal staphylococcal isolates was significantly higher in biofilm producing isolates compared to non-biofilm producing isolates and hence they concluded that staphylococcal isolates having biofilm propensity exhibit more resistance to antibiotics, hence are difficult to treat. Similarly, de Araujo et al.[34] reported that biofilm producing methicillin resistant staphylococcus isolates had a higher incidence of multi-resistance than biofilm non-producers from the same population. Also, Pramodhini et al.[35], in their study, showed significant correlation between biofilm production by *P. aeruginosa* and multidrug resistance where 80% of strains producing biofilm were multidrug resistant phenotypes.

**Conclusion:**

From this study we conclude that bacterial biofilm play an important role in the refractory otorrhea after TT insertion and *S. aureus* and *P. aeruginosa* biofilms are the commonest causes of PTTO making it resistant to medical treatment and necessitate removal of the TT to eradicate the bacterial biofilm and to treat ear infection.

**REFERENCES**


دور الأغشية الحيوية البكتيرية في حدوث التهاب الأنذ الپ،* هالة عبد المجيد طلبل**، حماده فضل هاشم*، محمد محمود إبراهيم**

كلية الطب - جامعة عين شمس

العنوان: يعتبر التجمع البكتيري السبب الرئيسي في ارتفاع معدلات التهاب الأنذ الپ المستمرة بدء إدراج أنزب فجر الطلبة مما يجعل التهاب الأنذ الپ لا يستجيب للعلاج باستخدام قطرات الذور الپ الموجودة.

الهدف من العمل: هو تحديد أنواع البكتيريا التي تتم على سطح أنزب فجر الطلبة و التي تتسبب في تشكيل التجمع البكتيري وتحديد جزيئات هذه الميكروبات الموجودة في الفم.

طريقة البحث: أجريت هذه الدراسة على 40 مرضىا يعانون من التهاب الذور الپ الويكي المصاحبة بالصاحب و تم علاجهم بواسطة أنزب فجر الطلبة تحت ملاحظة مستمرات و الفحص الكامل للثروة الأنذ الپي. وقد ظهر أن الذور الپ في 16 مراضا و تم أخذ عينات زراعة من الأنذ الخارجي والذور الپي و فحصتها و تحليلها.

تم إزالة الأنذ الپي في بعض الحالات للفحص الكامل للثروة. و تم إجراء تحليل بكتيري للثروة و استخراج مكوناتها و تحديد أساليب العلاج التي تستخدم في التحكم في التهاب الأنذ الپي.

استنتاج: تدل هذه الدراسة أن التجمع البكتيري في الثروة يلعب دورًا في حدوث التهاب الأذن الپي. و لذلك، يمكن استخدام العلاج الكيميائي و العلاج الفرعي للثروة لتفادي حدوث التهاب الأذن الپي. و من المهم أن يتم استخدام العلاج الفرعي للثروة و معالجة الأذن الپي بشكل صحيح.

و قد أظهرت الأبحاث النتائج التالية:

عدد 8 (من مجموع 12) من أنزب فجر الطلبة كانت بروفينج بسبب نجاعة شبه الكمية و كانت الميكروبات المقددة في الثروة.

أظهرت النتائج الأخرى أن الميكروبات المتعددة في الثروة مقددة بشكل فعال و أن أنزب فجر الطلبة قد يؤدي إلى حدوث التهاب الأذن الپي.

خاتمة: الدراسة نسستنتج أن استخدام الميكروبات المقددة في الثروة و استخدام العلاج الفرعي للثروة يمكن أن يؤدي إلى تقليل نجاعة شبه الكمية و منع حدوث التهاب الأذن الپي. و من المهم أن يتم استخدام العلاج الفرعي للثروة و معالجة الأذن الپي بشكل صحيح.

**دراسة في دورة العام 2012**

**المراجعات:**


