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Chronic Tonsillitis Bacteriology in Egyptian Children Including Antimicrobial Susceptibility

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Abstract: Objective: This study was conducted to analyze the underlying bacterial pathogens in tonsillar disease among Egyptian children, to investigate whether there is any difference between tonsillar surface and deep tissue cultures in patients who underwent tonsillectomy for recurrent tonsillitis and also to study the incidence of beta lactamase producing bacteria (βLPB) and Oxacillin resistant strains. Methods: Specimens were collected over a period of three months from different tonsillar sites of 72 patients –their ages ranging from 5 to 12 years - suffering from recurrent tonsillitis and undergoing tonsillectomy. Aerobic bacterial identification and antimicrobial susceptibility were carried out through standard procedures. Results: Sixty-five tonsils had positive aerobic isolates while the remaining seven cases had normal flora (9.7%). The total number of positive isolates in both surface and core cultures were 152. *S. aureus* was the most common aerobic organism (56.9%) isolated from both surface and core of excised tonsils. Most of *S. aureus* isolates (95%) had ß-Lactamase activity, whereas only 11% was Oxacillin resistant *S. aureus* (ORSA). *H. influenzae* was isolated in (44.6%) of cases, mostly as core cultures with 58% of them showing ß-Lactamase activity. GAβHS had an incidence of 38.5%, mostly as surface cultures with 26% of them showing ß-Lactamase activity. *Streptococcus pneumoniae* were isolated in 20% of cases, mostly surface isolates. *Klebsiella pneumoniae* were identified in the core of 5 cases (7.7%). There was a noticeable difference between surface and core cultures regarding all types of bacteria except S.aureus. Fifty percent discrepancy between the two sites was found. Conclusion: *S. aureus* is the main offending pathogen responsible for chronic tonsillitis nowadays. *H. influenzae* and GAβHS had a near equal incidence. Due to the difference between the isolates from tonsillar surface and core, the practice of surface tonsillar swab may be misleading. Tonsillar disease may stem from the bacteria within the substance of the tonsils rather than the bacteria identified on the surface.

Key words: Chronic tonsillitis, Bacteriology, Surface swab, Core culture, (βLPB), ORSA.

INTRODUCTION

Chronic tonsillitis refers to the condition in which there is enlargement of the tonsils accompanied by repeated attacks of infection. Tonsillitis is one the most common ailments encountered at childhood. Its incidence is especially high among children in the age group between 5 to 10 years. The inflamed tonsils harbor numerous types of bacteria, alone or in combination (Brook and Gober, 2006).

Antimicrobial treatment often fails to eradicate the pathogens and prevent recurrences of the tonsillar infection. Failure to eradicate pathogenic organisms in the core either due to inappropriate antimicrobial therapy or from inadequate antibiotic penetration in the core paves way to either persistence of core infection or reinoculation of the initially sterilized surface. If the surface culture is representative of the bacteriology of the core, then rational therapy could be directed at organisms cultured by surface swab (Kumar *et al*., 2005). This study was conducted to analyze the underlying bacterial pathogens in tonsillar disease, to investigate whether there is any difference between tonsillar surface and deep tissue cultures in patients who underwent tonsillectomy for recurrent tonsillitis and also to study the incidence of beta lactamase producing bacteria (βLPB) and Oxacillin resistant strains.

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MATERIAL AND METHODS

Patients & sampling: Specimens were collected over a period of three months from different tonsillar sites of 72 patients –their ages ranging from 5 to 12 years - suffering from recurrent tonsillitis and undergoing tonsillectomy. Fore each patient, specimens were taken aseptically from the surface (esp. from crypta magna before tonsillar removal) and deep tissue of the tonsils (after removal) using sterile swabs.

Bacteriological study: The swabs were inoculated (within 2 hours of collection) into Sheep blood (5 %), chocolate and MacConkey agar plates (Oxoid, Basingstoke, United Kingdom). Plates were incubated at 37 °C aerobically (MacConkey) and in 5% carbon dioxide (blood and chocolate), and examined at 24 and 48 h. Isolates were identified to genus level by conventional methods (Murray *et al*., 1995).

S. aureus identification was based upon colony & Gram stain morphologies and positive catalase reaction. A single colony of the isolate on the primary plate was subcultured onto Sheep blood agar (5%) to obtain a pure culture. Tube coagulase test for the detection of free coagulase activity on rabbit plasma (bio-Me´rieux, Marcy l'Etoile, France), and rapid slide latex agglutination tests (Slidex Staph Plus; bioMe´rieux) were performed for definitive identification of S.aureus.

Haemophilus influenzae identification was carried out by standard procedures including colony & Gram stain morphologies, biochemical reaction (oxidase and urease positive), requirement for X (hemin) and V (NAD) factors (Oxoid, Basingstoke, United Kingdom)(3). Group A β-haemolytic streptococci (GAβHS) were identified by colony & Gram stain morphologies, catalase test, and bacitracin sensitivity (Oxoid, Basingstoke, United Kingdom). *Streptococcus pneumoniae* isolates were identified by colony & Gram stain morphologies, catalase test, optochin-susceptibility (6 mm; Becton Dickinson Microbiology Systems) and Bile solubility using Deoxycholate reagent (2%; 0.5 mL; Becton Dickinson Microbiology Systems). *Klebsiella Pneumoniae* was identified by colony & Gram staining morphology and biochemical reactions using API 20E kit.

ß-Lactamase activity was determined for isolates by using Cefinase disks (Becton-Dickinson Microbiology Systems, Cockeysville, Md). Briefly, 6-mm-diameter filter paper disks impregnated with nitrocefin were moistened with 0.85% NaCl, and several well-isolated colonies of test organisms were transferred to the disk with a wire loop. Disks were examined for the appearance of a pink-red coloration for up to 60 min with isolates of S.aureus, and up to 10 min with the other species.

In order to detect Oxacillin resistant *S. aureus* (ORSA), *S. aureus* isolates were tested by disk diffusion using cefoxitin and Etest (AB Biodisk, Solna, Sweden) using cefoxitin and oxacillin. The inoculum was standardized to 0.5 McFarland turbidity. Disk diffusion was done with 10 µg and 30 µg disks (Oxoid, Basingstoke, United Kingdom) using Mueller-Hinton BBL II agar (Becton Dickinson, Heidelberg, Germany). Agar plates were incubated overnight (18 to 19 h) in ambient air at 37°C. Inhibition zone diameters were read from the back of the agar plate using reflected light and calipers to read to the nearest millimeter at the inner zone edge. For isolates with a zone size of >19 mm after 18 to 19 h for the 30 µg cefoxitin disk at 37°C, plates were further incubated and read after 24 h as specified in M100-S15.

S. aureus with a zone diameter of ≤ 19mm were scored as resistant and those with a zone diameter of ≥ 20mm were reported as susceptible. Etest oxacillin MIC testing was performed according to the manufacturer's instructions using Mueller-Hinton BBL II agar supplemented with 2% NaCl (wt/vol) and incubation at 37°C for a full 24 h. Etest cefoxitin MIC testing was done using Mueller-Hinton BBL II agar without NaCl supplementation. Plates were incubated in ambient air at 37°C. Cefoxitin MICs were read after 18 to 19 h of incubation.

RESULTS AND DISCUSSION

Results:

Total number of cases was 72 (47 boys – 25 girls). The age ranged between 5-12 years. Sixty-five tonsils had positive aerobic isolates while the remaining seven cases had normal flora (9.7%). The total number of positive isolates in both surface and core cultures were 152, as mixed types of bacteria were isolated in the same case. Thirty-four cases showed a single isolate in either surface or core cultures (whether they were the same or not), whereas the remaining 31 cases revealed mixed isolates. Analyzing the data of the present study we observed that *S. aureus* was the most common aerobic organisms encountered (56.9%). *H. influenzae* was the second isolate (44.6%), followed by GAβHS (38.5%). *Streptococcus pneumoniae* (20%) and *Klebsiella pneumoniae* (7.7%) were identified less frequently (Fig. 1).

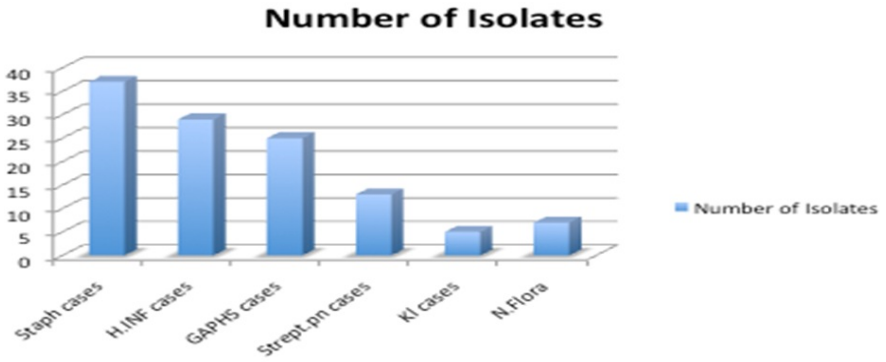

Fig. 1: Relation of different isolates.

As regards aerobic tonsillar bacteriology via surface swab, the total number was 72 isolates. *S. aureus* was the most common isolate followed by GAβHS. *H. influenzae* and *Streptococcus pneumoniae* were less frequently found on surface cultures than others. No *Klebsiella pneumoniae* was detected (Table 1)

Table 1: Types and number of isolates from the tonsillar surface.

Types of isolates	Number
S. aureus	26
H. influenzae	14
GAβHS	22
Streptococcal pneumoniae	10
Klebsiella pneumoniae	0
Normal flora	21

In aerobic tonsillar core bacteriology, the total number was 80 isolates. *S. aureus* was also the most common isolate followed by *H. influenzae* whereas GAβHS came in the third order. *Streptococcus pneumoniae* were also detected. *Klebsiella pneumoniae* was recovered from the core of 5 tonsils (Table 2)

Table 2: Types and number of positive isolates from the tonsillar core

Types	Number
S.aureus	31
H.influenzae	27
GAβHS	12
Streptococcal pneumoniae	5
Klebsiella pneumoniae	5
Normal flora	13

Correlation between types of isolates from tonsillar surface and core, there is a noticeable difference between the two sites regarding all types of bacteria except S.aureus. (Fig. 2)

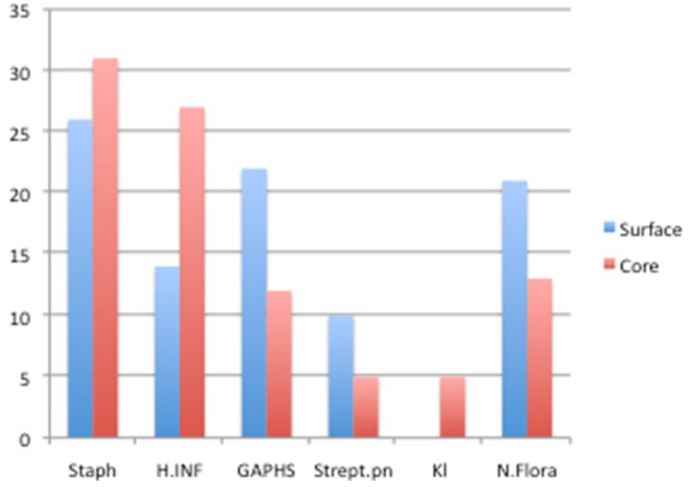

Fig. 2: Correlation between surface and core isolates

About half of cases (32), the isolates cultured from the surface differ from that of the core, whereas in the other half (33), the isolates from the surface were the same as recovered from the core. Twenty-six cases out of the later were identical (means that the isolates from the surface were the same as the isolates from the core), while in 7 cases there were additional types of bacteria either in surface or core (we termed these cases as similar but not identical) Table (3).

Table 3: Correlation between isolates from surface and core.

Identical isolates from surface & core	26
Similar isolates from surface & core	7
Different isolates between surface & core	32

Half of total number of the cultured isolates (76 out of 152) had ß-Lactamase activity, most of them were *S. aureus* followed by H.influenzae, and to a lesser extent GAβHS. (Table 4).

Table 4: Number of Beta lactamase producers.

Non producing	76
Producing:	76
S. aureus	54
H. influenzae	15
GAβHS	7

Most of *S. aureus* isolates (54 out of 57) had ß-Lactamase activity. More than half of *H. influenzae* (15 out of 26) had ß-Lactamase activity, whereas quarter of GAβHS (7 out of 27) had this activity (Fig. 3).

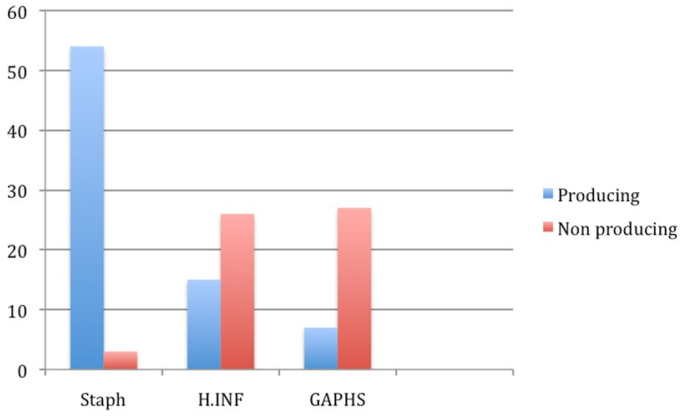

Fig. 3: Number of beta lactamase producers per type of isolates.

Regarding Oxacillin resistant *S. aureus* (ORSA), only 6 (out of 57) *S. aureus* were isolated.

Discussion:

Much has been written about bacteriology of recurrent tonsillitis but it remains a controversial topic. Effective treatment depends on knowledge of the infecting organisms. Despite the fact that tonsillitis is so common, consensus seems to be lacking as the main causative organisms and the differences between children and adults (Loganthana *et al*., 2006).

The present study revealed that *S. aureus* was the most common aerobic organism isolated from both surface and core of excised tonsils. This means that *S. aureus* is the main offending pathogen responsible for chronic tonsillitis nowadays. Most of *S. aureus* isolates (95%) had ß-Lactamase activity, whereas only 11% was Oxacillin resistant *S. aureus* (ORSA). *H. influenzae* was the second common isolate. Although it was less frequent in surface cultures than GAβHS, its prevalence in core cultures was much more than GAβHS. Fifty-eight percent of *H. influenzae* had ß-Lactamase activity. GAβHS came in the third order of frequency in this study; most of them were surface isolates. Twenty-six percent of them had ß-Lactamase activity. *Streptococcus pneumoniae* was isolated in 20% of cases, mostly surface isolates. *Klebsiella pneumoniae* were identified in the core of few cases.

This work matches with that of Jeong *et al*. (2007). They found that *S. aureus* was the most common pathogen (30.3% was isolated form the tonsillar core) in recurrent tonsillitis. After *S. aureus*, *H. influenzae* (15.5%) and GAβHS (14.4%) came in a near equal incidence. Mixed infection was common because of its high resistance to penicillin.

Many recent reports declared that *S. aureus* was the most common isolates in chronic tonsillitis (Kocaturk *et al*., 2003, Kumar *et al*., 2005, and Loganathan *et al*., 2006). What followed *S. aureus* was differently reported among workers. Kumar *et al*. (2005) arranged GAβHS, *Streptococcus pneumoniae* and then *H. influenzae* in a decreasing order.

Loganathan *et al*. (2006) stated that *S. aureus* was the most common pathogenic bacteria (40.9%) cultured both in adult and children. *H. influenzae* and GAβHS (23%) were more prevalent in children. *Streptococcus pneumoniae* and *Klebsiella pneumoniae* were more prevalent in adults. Kocaturk and his collagues (2003) concluded that *S. aureus* was the most common pathogen in core cultures of tonsils and adenoids. The frequency of ßLPB among isolated bacteria from tonsillar core was 44%. *S. aureus* was associated with betalactamase production in 88%.

Mahakit *et al*. (2005) reported that *H. influenzae* and *S. aureus* in the percentages of (25.9%) and (24%) were isolated from the crypts of 61 removed tonsils. Core cultures revealed *H. influenzae* (25.2%), *S. aureus* (23.4%) and Streptococcal Viridian (11.3%). Regarding ß-Lactamase production, 88.9% of *S. aureus* were b*et al* actamase-producing bacteria, while 29.2% of H. influenzae were ßLPB.

In a study done by Brook and Foote (2006), they cultured a total of 151 aerobic bacteria from the surface of 44 removed tonsils and 167 aerobic isolates from the core. The predominant isolates were alpha-hemolytic streptococci, GAβHS, S.aureus, gamma-hemolytic streptococci, *H. influenzae* and Moraxella catarrhalis. GAβHS and *S. aureus* were recovered more often from the core, where alpha-hemolytic streptococci and gamma-hemolytic streptococci were recovered from surface. Fifty-two percent ßLPB were recovered from tonsillar surface and 75% from core. The predominant ßLPB were S.aureus, *H. influenzae* and Moraxella catarrhalis. Seven isolates of MERSA were recovered from the cores and two were isolated from the surface (16%).

In our study, regarding correlation between types of isolates from tonsillar surface and core, there is a noticeable difference between the two sites regarding all types of bacteria except S.aureus. About half of cases (32), the isolates cultured from the surface differ from that of the core, whereas in the other half (33), the isolates from the surface were the same as recovered from the core. This means a 50% discrepancy between surface and core results. The surface of the tonsil is constantly exposed to oral secretions with their attendant flora and as such surface culture is likely to grow these organisms. Pathogen isolated from the surface culture may be colonizing the tonsil and not infecting it. Core organisms are responsible for pathological changes in the tonsils (Gaffney *et al*., 1991). This is correlated with our results where we found that 29% and 18% Normal Flora were isolated from the surface and core of tonsils respectively.

Kumar *et al*. (2005) reported that overall surface culture was in variance as the presence or absence of core pathogens in 58% cases, while in 42% was identical. Sixteen percent of cases of the study group showed normal flora. Gul *et al*. (2007) found that 52 out 77 patients; different types of bacteria were recovered from the surface and deep tissue cultures, whereas in 25 patients, the same types of bacteria were isolated from both surface and deep tissue cultures. The estimated probabilities of tonsillar bacteriology via surface swabs for H.influenzae, S.aureus, Streptococcal pneumoniae, and GAβHS were 27.2%, 38.4%, 66.6%, and 62.5% respectively. *H. influenzae* was less frequently predicted by surface cultures than others. So the authors thought that the swab cultures taken from the tonsillar surface might not always reveal the real pathogen of the tonsils. In addition, the estimated probability of tonsillar bacteriology by surface swabs varies with the type of the pathogen.

Meanwhile others proved that there was a high correlation in cultures obtained from tonsillar crypt and tonsillar core. Correlations were 100% specificity for GAβHS, 86.2% for *H. influenzae* and 81.5% for S.aurues. The difference in isolated technique may account for the higher correlation when compared to previous studies (Mahakit *et al*., 2005).

Conclusion:

S. aureus is the main offending pathogen responsible for chronic tonsillitis in Egyptian children nowadays. Most of *S. aureus* isolates (95%) had ß-Lactamase activity, whereas only 11% was Oxacillin resistant *S. aureus* (ORSA). *H. influenzae* and GAβHS had a near equal incidence. *H. influenzae* was less frequent in surface cultures than GAβHS, yet; its prevalence in core cultures was much more than GAβHS. More than half of *H. influenzae* had ß-Lactamase activity, whereas about quarter of GAβHS had this activity. *Streptococcus pneumoniae* were isolated in 20% of cases, mostly surface isolates. *Klebsiella pneumoniae* were identified in

the core of few cases. There was a noticeable difference between the isolates from tonsillar surface and core regarding all types of bacteria except *S. aureus*. The practice of surface tonsillar swab may be misleading. Tonsillar disease may stem from the bacteria within the substance of the tonsils rather than the bacteria identified on the surface.

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