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Original Research Article

Evaluation of Surface and Core Bacterial Isolates from Adenotonsillar Tissue in Nigerian Children

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Abstract: ***Background*:** Adenoid and tonsils which play a protective role against infections in healthy individuals may become a reservoir for pathogenic microorganisms. Infection of the adenoid and palatine tonsils continue to be a major burden among children in our environment**.** Bacteria colonize the surface and core of the adenoid and tonsils. In University of Port-Harcourt Teaching Hospital, there is a practice of taking surface swab of palatine tonsils for microscopy, culture and sensitivity to guide antimicrobial therapy which may not reflect the core pathogens. There may be a difference between surface and core aerobic bacteriology of adenoid and tonsils hence this study. ***Aim***: To compare surface versus core aerobic bacteria of adenoid and tonsillar tissues in children undergoing adenotonsillectomy at University of Port-Harcourt Teaching Hospital. ***Materials and Methods*:** This was a hospital based cross-sectional study involving fifty(50) paediatric participants aged 3-7years undergoing adenotonsillectomy. Informed consent was sought from patient's caregiver and a pretested proforma was used to recruit the participants for the study. Surface swabs of the tonsils and adenoid were taken following general anaesthesia and orotracheal intubation in the theatre. The core tissue specimens of adenoid and tonsils were collected after adenotonsillectomy. The core specimens and surface swabs were sent immediately to microbiology laboratory for processing. Isolation of bacteria by Gram staining and biochemical testing was done, followed by susceptibility testing and beta-lactamase production detection. Results were analyzed using the Statistical Product and Service Solutions (SPSS) version 20. Pearson's chi square statistics was used to determine the difference in the bacteriology between the surface and core of the adenoid and tonsils. A p-value of less than 0.05 was considered statistically significant. ***Results*:** In the demographic characteristics of the study group, age range less than 5 years had the highest number of adenotonsillectomy (62.0%) and males (72.0%) were more than females (28.0%). Snoring (94.0%) accounted for the commonest symptom, followed by mouth breathing (84.0%) among the participants in this study. The surface and core bacterial pattern of adenoid was predominated by *Staphylococcus aureus.* The surface of the tonsil was predominated by viridans group of *Streptococcus* while *Staphylococcus aureus* was commonest in the core of the tonsils. ***Conclusion*:** The organisms isolated from the surface of adenoid and tonsils were similar to those in the core tissues. Therefore, surface swabs may predict core pathogenic organisms in tonsillitis and adenoiditis.

Keywords: Adenoid, Tonsils, Bacteriology, Surface and Core.

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INTRODUCTION

Aerobic bacteria commonly cultured from the tonsils and adenoid are *Group A beta-hemolytic Streptococci, Group B, C, G Streptococci, Haemophilus influenza, Streptococcus pneumonia, Moraxella catarrhalis, Staphylococcus aureus, Haemophilus parainfluenza, Neisseria spp* and *Mycobacteria spp* [1]. There is acceptance of the concept that the disruption in normal bacterial homeostasis of the upper aerodigestive tract with shifting of commensals to potential pathogens result from recurrent infection by viral agents and beta-lactamase producing organisms [1, 2]. *Staphylococcus aureus, Haemophilus spp* and *Streptococcus spp* persist predominantly intracellular within mucosal biofilms in the adenoid and tonsillar tissues [3]. This mucosal biofilm formation has been reported as an important factor in the chronicity of infection of adenoid and tonsils [3]. Another factor implicated in chronic tonsillar infection is crypt obstruction resulting in bacterial stasis [1].

Adenoid and palatine tonsils represent the primary defence against microbes entering the upper aerodigestive tract. During childhood there is enlargement of adenotonsillar tissue from increased activity brought about by recurrent upper respiratory tract inflammation. Study has shown that bacteria were associated with most upper respiratory tract infection (URTI) in under 5years [4]. These bacteria has been suggested in the etiology of adenotonsillar hyperplasia with size of adenoid/tonsil directly proportional to the aerobic bacterial load and absolute number of lymphocytes [5]. The concentration of bacteria correlates with clinical parameters of infection and hyperplasia of tonsils [6]. In recurrent tonsillitis, tonsillar enlargement from follicular hyperplasia has been reported [7]. Allergy has also been implicated as an important risk factor for adenotonsillar hypertrophy [8].

Enlargement of adenoid and tonsils causes narrowing of pharyngeal airway which results in snoring, mouth breathing, nasal blockage, nasal discharge, apnoiec attacks amongst other symptoms. Snoring was reported to be the commonest presentation [9] and has a prevalence of 27.9% in Calabar [10]. There is a significant correlation between the symptomatology score and the age, with younger children between 2-6 years having more severe symptoms than the older ones [11, 12]. Olagunju *et al*., in South Western Nigeria reported children with obstructive adenotonsillar hypertrophy to have a mean age of 36.7months [13]. Similarly, age group 3-5 years was also reported to have the highest number of adenotonsillectomy in Port-Harcourt [14].

Adenoid and tonsils which play a protective role against infections in healthy individuals may become a reservoir for pathogenic microorganisms [15]. There is colonization of the surface and the core adenoid and tonsils by pathogenic bacteria [16, 17]. *Streptococcus pyogenes* is the most commonly identified organism from the surface of the tonsils in disease [18]. It represents the most frequent bacterial aetiological agent of paediatric upper respiratory tract infection (URTI) [19]. In Benin city, *Beta-hemolytic streptococcus* was reported as the commonest cause of tonsillopharyngitis in children with a prevalence of 48.72% [20].

Infection of the adenoid and palatine tonsils continue to be a major burden among children in our environment [21]. Besides *Streptococcus pyogenes*, some atypical bacteria may be responsible for recurrent infection of adenoid and tonsils [19]. There is domination of beta–lactamase producing aerobes, facultative bacteria [22] and biofilm-producing bacteria (BPB) such as *Staphylococcus aureus* in recurrent tonsillitis [23]. Generally, tonsillar surface culture report is used as a guide for antimicrobial therapy. However, tonsillar infection may stem from the bacteria within the core of the tonsil rather than the surface [24]. This could cause failure of antibiotic therapy because pathogens from the deep tissue are not isolated thus leading to recurrence and chronicity. Hence, the need to determine and compare the surface and core aerobic bacteria of adenoid and tonsillar tissues in children undergoing adenotonsillectomy at University of Port-Harcourt Teaching Hospital.

Recurrent and chronic tonsillitis is a global public health issue which can severely impair an individual's quality of life [25] and is a significant burden on public health [26]. It has resulted in school absenteeism among children [27]. There is affectation of healthy growth and development in children from recurrent symptoms. Adenoid as a reservoir for bacteria may cause adenotonsillitis, middle ear infections and rhinosinusitis [28]. Adenotonsillitis is the highest single disorder encountered in pediatric age group in ORL practice in Port-Harcourt with a prevalence of 18.6% [29]. The prevalence of bacterial tonsillitis caused by *Streptococcus pyogenes* was found to be 17% among children aged 1-5 years in Awka [30], Nigeria. Recurrent adenoid and tonsillar infection is the second commonest indication for adenotonsillectomy [31]. Despite the widespread use of antibiotics, adenotonsillitis is often recalcitrant and recurrent leading to frustration by parents and medical staff, which finally ends in decision for surgery [3]. Caregivers incur huge financial expenses from recurrent hospital visits through direct payment [31].

MATERIALS AND METHODS

This is a hospital based cross-sectional study involving children undergoing adenotonsillectomy, comparing the aerobic surface and core bacteriology of the adenoid and tonsils.The study population comprised all the new patients aged 3-7 years seen at the ORL clinic of UPTH within the study period. All children aged 3-7 years undergoing adenotonsillectomy at UPTH. Children

who are on antibiotics a week or less prior to scheduling adenotonsillectomy and Children whose caregivers did not give consent were excluded from study. Hence, a surface swab each was taken from the adenoid, right tonsil and left tonsil. After adenotonsillectomy, a core tissue specimen was also taken from the adenoid, right tonsil and left tonsil of 50 participants in this study.

Six samples from each participant which gave a total of 300 samples analyzed for this study. Consecutive sampling technique was used to recruit patients that met the inclusion criteria. Participants for this study were recruited as they were admitted into ORL ward of UPTH for adenotonsillectomy. Health Education on the objectives of the study was given to the caregivers and understood, informed consent taken.In theatre, after general anaesthesia and endotracheal intubation with sterile endotracheal tube.The mouth was then opened and oropharynx exposed with mouth gag after positioning the patient. A surface swab was taken from right and left tonsil by rotating a sterile cotton wool swab stick on each of the tonsils while avoiding contact with other parts of the oropharynx. The same procedure was done on the surface of the adenoid after retracting the soft palate backward with pillar retractor. Adenoid tissue was shaved off by gentle sweeping movement of engaged proper size of Beckmann adenoid curette without guard. The right tonsil was pulled medially with Dennis-Brown tonsil holding forceps and incision was made in the mucous membrane where it reflects from the tonsil to the anterior pillar. This was extended with scissors and tonsil was dissected with Gwynne-Evan tonsillar dissector from the upper pole until lower pole was reached and then snared with Eve's tonsillar snare. Sterile suction tube was used in maintaining a clear field without blood throughout the procedure. The same procedure was done for the left tonsil. Each excised tissue was dipped into 10% povidone iodine solution for about 30 seconds and thoroughly rinsed with sterile normal saline. Thereafter, each tissue was cut opened with sterile blade and part of the core tissue excised. Surface swabs were put into the swab jackets and core specimens were carried in universal containers. Specimens were labeled appropriately and transported immediately to microbiology laboratory of UPTH for further processing.

Specimen processing involved: Inoculation of the samples on chocolate, Mac-Conkey, gentamycin blood and crystal violet blood agars. The plates were incubated aerobically at 35-37ºC for 24-48 hours. This was done with the assistance of the laboratory scientists. After 24-48 hours of incubation, plates were examined for evidence of growth of organisms. Thereafter, Gram staining of the colonies was done to ascertain the morphological features and further biochemical characteristics were determined using the following tests: oxidase, coagulase, citrate utilization, lactose fermentation, catalase, urease and indole production. Bacterial isolates were identified by comparing their characteristics with those of known taxonomy [32, 33]. Lancefield grouping, bacitracin and optochin sensitivity testing were done for *Streptococci spp*. Susceptibility testing was done by disk diffusion method. Oxoid antibiotics susceptibility test discs used includes: 5mcg ciprofloxacin(CIP), 10mcg gentamycin(GN), 30mcg ceftazidime(CAZ), ceftriaxone(CRO), 10mcg penicillin(P), 30mcg cefuroxime(CXM), 10mcg amoxicillin/clavulanic acid(AMC) and 15mcg erythromycin(E). The diffusion zone diameters were calculated and compared with standard reference using Clinical and Laboratory Standards Institute method(CLSI) [34]. Acidometric filter paper test for beta-lactamase activity was done for the isolated organisms. A change in colour of the bromocresol purple indicator from purple to yellow was taken as positive for beta-lactamase production.

Data entry and analysis was done using the Statistical Product and Service Solutions (SPSS) version 20. Tables and charts were used as appropriate to represent data. Numerical variables were summarized as means and standard deviation for age while categorical variables were expressed as frequencies and percentages. Pearson's chi-square statistics was used to determine the difference in the bacteriology between the surface and core of the adenoid and tonsils. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic Characteristics of Study Group

A total of 50 paediatric participants which met the inclusion criteria were included in the study. The age ranges from 3 years to 7 years. From this study, a median age of 4.0 years and mean age of 4.4± 1.5 years was got. As seen in Table 1, age group less than 5 years had the highest number of adenotonsillectomy accounting for 62.0% and greater than or equal 5 years accounted for 38.0%. Males made up 72.0% while females accounted for 28.0% with a M:F=1.3:1.

Table 1: Demographic characteristics of study group (n=50)

	N	%
Age group		
<5 years	31	62.0
≥5 years	19	38.0
Gender		
Female	14	28.0
Male	36	72.0
Total	**50**	**100.0**

Surface and Core Bacteria of Adenoid

Staphylococcus aureus was the commonest isolates from the surface and core of the adenoid. There was no statistically significant difference between the surface bacteriology and the core bacteriology of adenoid (p-value=0.525) as depicted in Table 2.

Table 2: Aerobic surface and core bacteria of adenoid

Organisms	Adenoid			
	Surface		Core	
	N	%	N	%
No growth	20	40.0	10	20.0
Viridans group of *Streptococcus*	2	4.0	3	6.0
Streptococcus pyogenes	2	4.0	4	8.0
Citrobacter spp	1	2.0	0	0.0
Haemophilus spp	3	6.0	5	10.0
Escherichia coli	4	8.0	6	12.0
Klebsiella spp	2	4.0	1	2.0
Pseudomonas aeruginosa	5	10.0	7	14.0
Staphyloccoccus aureus	11	22.0	14	28.0
Total	**50**	**100.0**	**50**	**100.0**

Chi-square = 7.13, Fishers exact p-value = 0.525

Surface and Core Bacteria of Tonsils

While viridans group of *Streptococcus* was predominant on the surface, *Staphylococcus aureus* was the commonest organism in the core of the tonsils. There was no statistically significant difference between the surface bacteria of the right and left tonsil (p-value=0.992), core bacteria of the right and left tonsil (p-value=0.910) and the surface and core of the tonsils (p-value=0.628) as seen in Tables 3 & 4.

Table 3: Aerobic surface bacteria of tonsils

Organisms	Surface			
	Right tonsil		Left tonsil	
	N	%	N	%
No growth	16	32.0	15	30.0
Viridan group of Streptococcus	9	18.0	10	20.0
Streptococcus pyogenes	3	6.0	3	6.0
Haemophilus spp	4	8.0	3	6.0
Escherichia coli	4	8.0	5	10.0
Klebsiella spp	5	10.0	3	6.0
Pseudomonas aeruginosa	3	6.0	3	6.0
Staphyloccoccus aureus	6	12.0	8	16.0
Total	**50**	**100.0**	**50**	**100.0**

Chi-square = 1.12, Fishers exact p-value = 0.992

Table 4: Aerobic core bacteria of tonsils

Organisms	Core			
	Right tonsil		Left tonsil	
	N	%	N	%
No growth	14	28.0	10	20.0
Viridan group of Streptococcus	7	14.0	5	10.0
Streptococcus pyogenes	3	6.0	2	4.0
Haemophylus spp	4	8.0	6	12.0
Escherichia coli	4	8.0	6	12.0
Klebsiella spp	5	10.0	4	8.0
Pseudomonas aeruginosa	4	8.0	6	12.0
Staphyloccoccus aureus	9	18.0	11	22.0
Total	**50**	**100.0**	**50**	**100.0**

Chi-square =2.17, Fishers exact p-value =0.910

DISCUSSION

The most predominant symptom of the participants undergoing adenotonsillectomy in this study was snoring, followed by mouth breathing which agrees with the reports of similar studies on adenotonsillectomy in children by Mbam *et al.,* [12], Adegbiji *et al.,* [35] and Samdi *et al.,* [36]. In contrast, nasal obstruction, noisy breathing and mouth breathing were the commonest symptoms, followed by snoring in a similar study by Adedeji *et al* in Osogbo [37].

In this study, *Staphylococcus aureus* was the most prevalent aerobe isolated from the adenoid surface

and core. This agrees with Rasha *et al.*, [38] in Iraq on aerobic bacterial profile associated with chronic tonsillitis and adenoid hypertrophy in children who reported *Staphylococcus aureus* as one of the predominant organisms on the surface of the adenoid. However, *Escherichia coli* was found to be the most prevalent isolate in the core of adenoid tissue [39]. This is probably because *Staphylococcus aureus* persists within mucosal biofilms in the adenotonsillar tissues [3] and thus resistant to routine antibiotics. Rajeshwary *et al.*, [40] in India found mainly commensals on the surface but aerobic core bacterial pattern was dominated by *Staphylococcus aureus.* There were no bacteria isolated in 40% of surface samples and 20% of core samples of adenoid and this could be attributed to fastidious organisms.

In this study, there was no statistically significant difference between the aerobic surface bacteriology and core bacteriology of the adenoid (p=0.525). This is similar to other findings [41-43]. This could have resulted from migration of organisms between the adenoidal sites. However, it differs from Taylan *et al.*, in Turkey who found statistically significant difference between the surface and core bacteria of adenoid.

Regarding aerobic organisms on the tonsillar surface, viridans group of S*treptococcus* was most prevalent in this study. In contrast, *Staphylococcus aureus* accounted for the most predominant isolates of surface throat swab in a study on microbiological profile of pharyngotonsillitis in National Hospital, Abuja, Nigeria [45]. Viridans group of *Streptococcus* is part of the normal flora of the oropharynx and there is usually contamination of tonsillar surface with oropharyngeal secretions [46]. This may be linked to its prevalence on the surface of the tonsils. The isolation of gram negative coliforms such *Pseudomonas aeruginosa* and *Klebsiella spp* may be attributed to poor hygiene among the children. This is different from the findings of Okoye *et al.*, [47] in Awka and Garbe *et al* in Gusau who reported *Streptococcus pyogenes* as the most prevalent pathogen implicated in pharyngotonsillitis. Pathogenic organisms may vary from one geographical location to another.

In the tonsillar core, *Staphylococcus aureus* was the most prevalent pathogen. Similarly, in Benin city, south-south Nigeria, a study on bacterial tonsillar microbiota and antibiogram in recurrent tonsillitis revealed *Staphylococcus aureus* as the commonest isolate from the tonsillar core tissue [47]. Contrary to this, *Klebsiella pneumonia* was reported by Rasha *et al.*, [39] to be predominant in the core of the tonsils. There were no bacteria isolated in 31% of surface samples and 24% of core samples of tonsils and the reason may not be different from that of the adenoid.

In this study, there was no statistically significant difference between the surface and core bacteriology of tonsils (p=0.628). This is in agreement with Douglas *et al.*, [48] in Ibadan and other works [49, 50]. The reason may not be different from that stated for adenoid. On the contrary, Babaiwa *et al.*, [47] in Benin city reported a significant difference in bacterial isolates obtained from the tonsillar core compared with that on the surface among his subjects and similar to other findings [51, 52].

CONCLUSION

The aerobic surface bacterial pattern of the adenoid was predominated by *Staphylococcus aureus* while that of tonsils was predominated by viridans group of *Streptococcus.*

The aerobic core bacterial pattern of adenoid and tonsils was predominated by *Staphylococcus aureus.*

There was no statistically significant difference between the aerobic surface and core bacteriology of adenoid and tonsils

RECOMMENDATION

1. Surface swab culture of the palatine tonsils were satisfactory and therefore should be continued.
2. Further research should be carried out compare the anaerobic isolates of surface and core tonsils.

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