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Original Research Article

Characterization and frequency of biofilms in adenotonsillitis: a retrospective study from a tertiary hospital in North-Eastern Nigeria

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ABSTRACT

Background: Adenotonsillitis, a common condition characterized by inflammation of the adenoids and tonsils, is caused by bacterial and fungal pathogens. Biofilm formation has been linked to disease chronicity and antibiotic resistance. However, the role of biofilms in adenotonsillitis remains poorly understood. This study aims to explore biofilms in adenotonsillitis biopsies, focusing on their characterization, frequency, and demographic distribution by determining the expression of polysaccharides in the biofilm matrix using Congo red stain, determining the presence and frequency of bacterial as well as fungal biofilms in adenotonsillar tissue, investigating any potential associations with disease severity, and evaluating the age and sex distribution of patients with adenotonsillitis.
Methods: This retrospective study analyzed formalin-fixed paraffin-embedded adenotonsillitis biopsies (n=50) collected from the university of Maiduguri teaching hospital. The expression of polysaccharides in the biofilm matrix was assessed using congo red stain. Bacterial and fungal biofilms were visualized using crystal violet and Gomori methenamine silver (GMS) stains, respectively. Data on patient demographics, diagnoses, and biofilm characteristics were analyzed.
Results: Adenoidtonsillitis was the most common diagnosis (82%), predominantly affecting children aged 0-9 years (76%). Gram's reaction was positive in 70% of cases, while Congo red staining indicated polysaccharide expression in 60%. GMS staining revealed fungal elements in 18% of cases.
Conclusions: This study sheds light on the characterization and frequency of bacterial and fungal biofilms in adenotonsillitis, emphasizing importance of biofilms in disease development and persistence. Understanding biofilm-associated infections can improve diagnostic and treatment strategies for adenotonsillitis in Nigeria and beyond

Keywords: Adenotonsillitis, Bacterial biofilm, Fungal biofilm, Congo red stain, GMS stain

INTRODUCTION

Adenotonsillitis, characterized by inflammation of the adenoids and tonsils, is a common condition affecting individuals of all age groups.[1] It is primarily caused by bacterial and fungal pathogens, and recent research has shed light on the role of biofilm formation in the pathogenesis and chronicity of the disease.[2,3] Biofilms are complex microbial communities encased in a self-produced extracellular matrix, rendering them highly resistant to antibiotics and host immune responses.[4]

Despite advancements in our understanding of adenotonsillitis, the role of biofilms in disease

development and persistence remains poorly understood. Biofilms have been implicated in chronic and recurrent infections, posing significant challenges for effective treatment.[4] Understanding the characteristics, frequency, and demographics of biofilms in adenotonsillitis can provide valuable insights into disease progression, diagnostic challenges, and treatment strategies. This study aims to explore biofilms in adenotonsillitis biopsies, focusing on their characterization, frequency, and demographic distribution by determining the expression of polysaccharides in the biofilm matrix using congo red stain, determining the presence and frequency of bacterial as well as fungal biofilms in adenotonsillar tissue and investigate any potential associations with disease severity and investigating the age and sex distribution of patients with adenotonsillitis and evaluate any potential associations with the occurrence of bacterial and fungal biofilms.

METHODS

Study design

This retrospective study utilized formalin-fixed paraffin-embedded adenotonsillitis biopsies collected from the university of Maiduguri teaching hospital between January 2022 and December 2022.

Ethical considerations

Ethical approval was obtained from the institutional review board (IRB) of the university of Maiduguri teaching hospital prior to the commencement of the study. Patient confidentiality and data protection were strictly maintained throughout the study. All procedures were conducted in accordance with the ethical guidelines outlined in the declaration of Helsinki.[5]

Sample collection

A total of 50 adenotonsillitis biopsy samples were included in the analysis. The biopsy specimens were obtained from patients who presented with clinical symptoms and underwent adenotonsillectomy as part of their standard medical care. The samples were collected during the specified study period and stored as formalin-fixed paraffin-embedded tissue blocks.

Inclusion and exclusion criteria

The inclusion criteria involved formalin fixed paraffin embedded tissue samples received between January 2022 and December 2022 retrieved from histopathology archive. Furthermore, the study included only those tissue samples with adequate preservation.

Conversely, formalin fixed, paraffin embedded histopathological samples other than adenotonsillar tissued were excluded. Similarly, samples lacking comprehensive histopathological data or falling outside the designated study period were not considered. The unavailability of tissue samples within the histopathology archive also led to exclusion. Additionally, tissue samples exhibiting inadequate quality or severe degradation, which could potentially compromise accurate staining and analysis, were excluded from the study too.

Staining techniques

Polysaccharide expression: Congo red stain was employed to assess the expression of polysaccharides within biofilm matrix.[6] Formalin-fixed paraffin-embedded tissue sections deparaffinized and rehydrated. The sections were then treated with congo red dye for a specified duration. Afterward, the sections were rinsed to remove any unbound dye and examined under light microscope. Presence of congo red staining indicated expression of polysaccharides in biofilm matrix.

Bacterial biofilm: Crystal violet stain was used to visualize bacterial biofilms.[7] Sections of the formalin-fixed paraffin-embedded adenotonsillitis biopsies were deparaffinized and rehydrated. The tissue sections were then incubated with crystal violet dye for a specific period of time. Subsequently, the sections were rinsed to remove excess dye and observed under light microscope. The presence of stained biofilms indicated the presence of bacterial biofilms in the adenotonsillar tissue.

Fungal biofilm: GMS stain was used to detect fungal biofilms.[8] The tissue sections were deparaffinized and rehydrated, followed by treatment with GMS stain. After the specified incubation period, the sections were rinsed to remove excess stain and observed under a light microscope. The presence of stained structures indicated the presence of fungal biofilms in adenotonsillar tissue.

Data collection

Microscopic analysis was performed by experienced pathologists to evaluate the presence and distribution of bacterial and fungal biofilms in the adenotonsillitis biopsies. The presence of bacterial biofilms, as demonstrated by crystal violet stain, the expression of polysaccharides using congo red stain, and the presence of fungal biofilms using GMS stain were recorded for each biopsy sample.

Data analysis

Descriptive statistics were used to analyze the frequency of bacterial and fungal biofilms in the adenotonsillitis biopsy samples. The age and sex distribution of patients with adenotonsillitis were also determined through the examination of patient records. The collected data on the presence and characterization of bacterial and fungal biofilms, as well as the age and sex distribution of patients with adenotonsillitis, were analyzed using SPSS version 20.0 statistical package. The frequency of bacterial and fungal biofilms in the adenotonsillitis

biopsy samples was determined, providing insights into the prevalence of biofilm-associated infections.

RESULTS

Table 1 presents the age and gender distribution of adenotonsillectomy cases. In the age range of highest cases were seen in the age group 0-9 years with 19 male cases and 19 female cases, making a total of 38 cases, which represent 76% of the total cases.

Table 1: Age and gender distribution of adenotonsillectomy cases.

Age range (In years)	Gender		Total	Percent (%)
	Male	Female		
0-9	19	19	38	76
10-19	2	4	6	12
20-29	0	6	6	12
Total	21	29	50	100

Table 2 shows the distribution of diagnoses in adenotonsillectomy cases. Adenotonsillitis was the most common diagnosis, accounting for 41 cases and representing 82% of the total cases. While adenoid follicular hyperplasia was the least with 2 cases, making up 4% of the total cases.

Table 2: Distribution of diagnoses in adenotonsillectomy cases.

Diagnosis	Frequency	Percent (%)
Adenotonsilitis	41	82.0
Adenotonsilar lymphoid hyperplasia	7	14.0
Adenoid follicular hyperplasia	2	4.0
Total	50	100.0

Table 3 displays the results of Gram's reaction in adenotonsillectomy cases. Out of the total cases included in the study, Gram's reaction was positive in 35 cases, constituting 70% of the cases. Gram's reaction was negative in 15 cases, making up 30% of the total cases.

Table 3: Gram's reaction results in adenotonsillitis cases.

Predorminant Gram's reaction	Frequency	Percent (%)
Positive	35	70
Negative	15	30
Total	50	100

Table 4 showcases the results of Congo red staining in adenotonsillectomy cases. Out of the total cases included in the study, Congo red staining was positive in 30 cases, constituting 60% of the cases.

Table 4: Congo red staining results in adenotonsillectomy cases.

Congo red	Frequency	Percent (%)
Positive	30	60
Negative	20	40
Total	50	100

Table 5 displays the results of GMS staining in adenotonsillectomy cases. Out of the total cases included in the study, GMS staining was positive in 9 cases, comprising 18% of the cases. GMS staining was negative in 41 cases, making up 82% of the total cases.

Table 5: GMS staining results in adenotonsillectomy cases.

GMS	Frequency	Percent (%)
Positive	9	18
Negative	41	82
Total	50	100

Table 6 provides the distribution of Gram's reaction in different conditions of adenotonsillectomy diagnosed. Among the cases diagnosed as Adenotonsillitis, 29 cases exhibited a positive Gram's reaction, while 12 cases showed a negative Gram's reaction. In cases of adenoid tonsillar tissue lymphoid hyperplasia, 4 cases had a positive Gram's reaction, while 3 cases had a negative Gram's reaction. Both cases of adenoid follicular hyperplasia showed a positive Gram's reaction.

Table 6: Gram's reaction in different laboratory diagnosed adenotonsillectomy.

Clinical condition	Gram's reaction		Total
	Positive	Negative	
Adenoidtonsilitis	29	12	41
Adenotonsilar lymphoid hyperplasia	4	3	7
Adenoid follicular hyperplasia	2	0	2
Total	35	15	50

Table 7 displays the distribution of Congo red staining results in adenotonsillectomy cases. Among the cases diagnosed as adenotonsillitis exhibited the highest expression of biofilm matrix with 24 cases positive Congo red staining result.

Table 8 illustrates the distribution of (Grocott GMS) staining results in different clinical conditions of adenotonsillectomy. Out of the cases diagnosed as Adenotonsillitis, 8 cases exhibited a positive GMS staining result, while 33 cases showed a negative result.

Table 9 displays distribution of adenotonsillectomy patients address categorized as within metropolis/ outside metropolis. All cases originated from within metropolis.

Table 7: Congo red staining results in different clinical conditions of adenotonsillitis.

Clinical condition	Congo red		Total
	Positive	Negative	
Adenoidtonsilitis	24	17	41
Adenotonsilar lymphoid hyperplasia	4	3	7
Adenoid follicular hyperplasia	2	0	2
Total	30	20	50

Table 8: GMS staining results in different clinical conditions of adenotonsillitis.

Clinical condition	GMS		Total
	Positive	Negative	
Adenoidtonsilitis	8	33	41
Adenotonsilar lymphoid hyperplasia	1	6	7
Adenoid follicular hyperplasia	0	2	2
Total	9	41	50

Table 9: Distribution of adenotonsillectomy based on address.

Clinical condition	Address		Total
	Within metropolis	Outside metropolis	
Adenoidtonsilitis	41	0	41
Adenotonsilar lymphoid hyperplasia	7	0	7
Adenoid follicular hyperplasia	2	0	2
Total	50	0	50

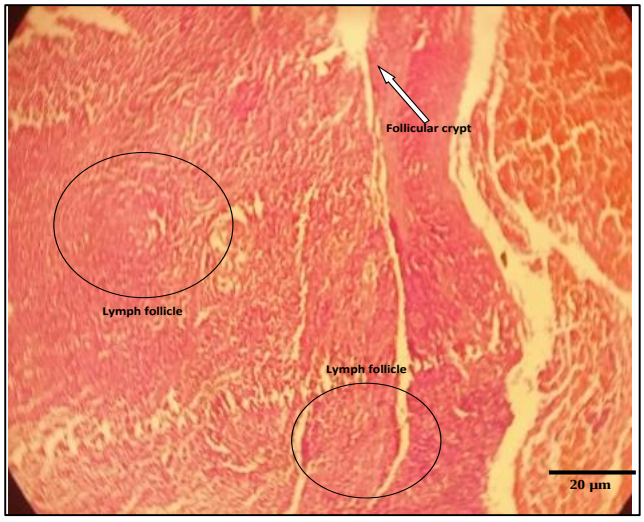

Figure 1: Hematoxylin and eosin stain, showing adenotonsillitis tissue (bar=50 microns).

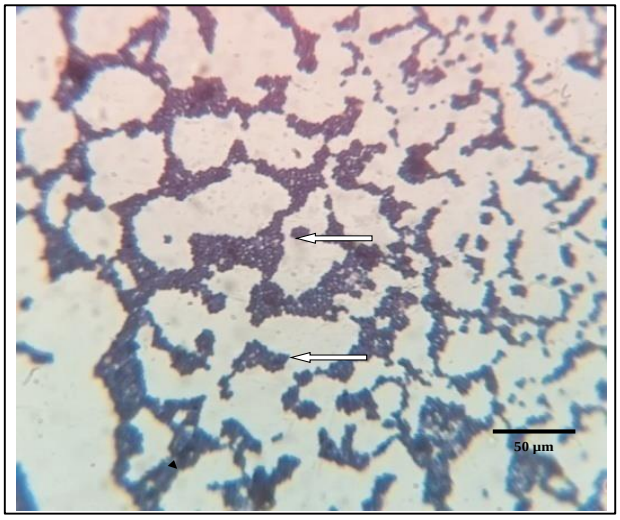

Figure 2: Gram's stain, demonstrating gram positive coccus bacteria in clusters (Control) (bar=50 microns).

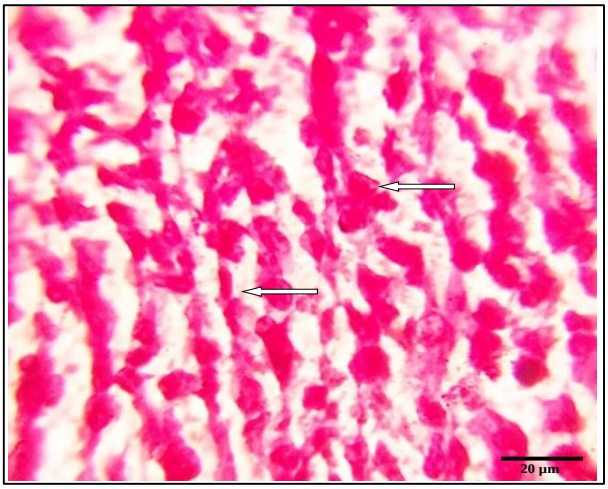

Figure 3: Gram's stain, demonstrating gram negative bacillus bacteria in clusters in adenotonsillitis tissue (bar=20 microns).

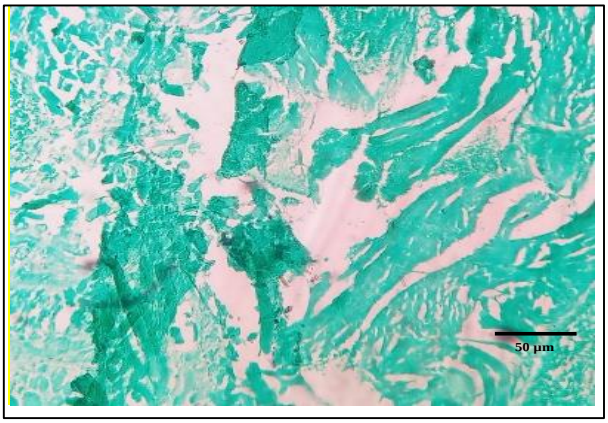

Figure 4: GMS stain, demonstrating normal adenotonsillar tissue, no fungal hyphae or spores (Negative control, bar=50 microns).

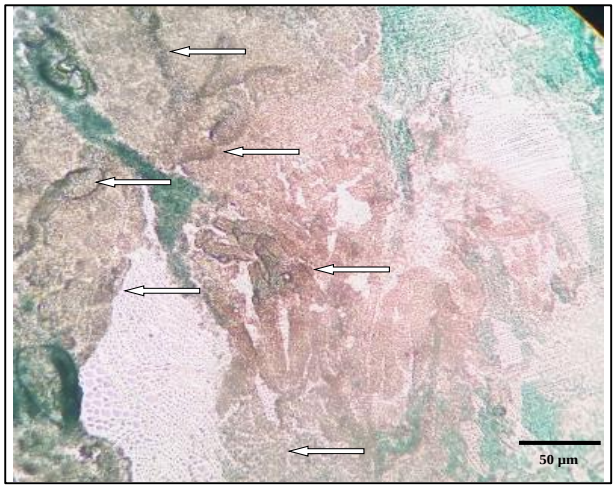

Figure 5: Fungal spores demonstrated by the Grocott methenamine silver special stain in adenotonsillar tissue with adenotonsillitis.

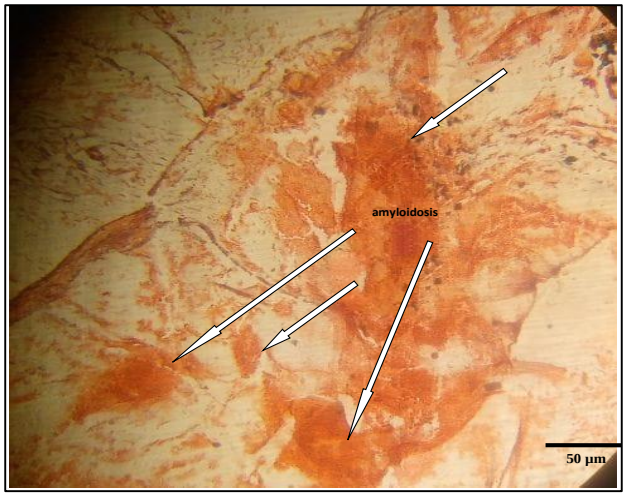

Figure 6: Congo red reaction, demonstrating amyloids (Control) (bar=50 microns).

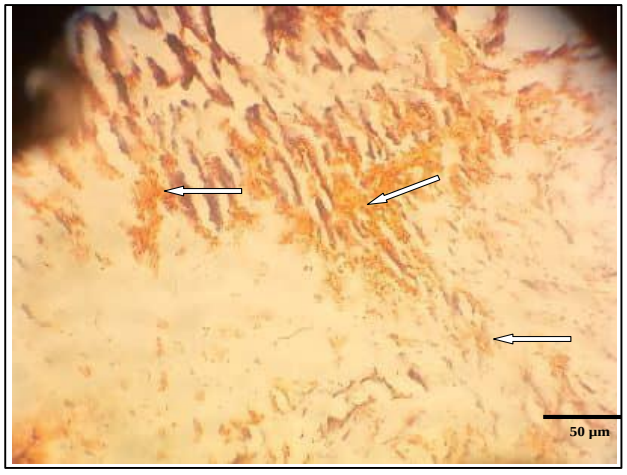

Figure 7: Adenotonsillitis tissue stained with congo red reaction reveals clear distribution of biofilm matrix against clean background (bar=50 microns).

DISCUSSION

The results of this study provide valuable insights into the age and gender distribution, diagnoses, and laboratory findings in cases of adenotonsillitis. The study included a total of 50 cases, with different age ranges and genders represented. In the age range of 0-9 years, both male and female cases were equally distributed, accounting for the majority (76%) of the total cases. This finding is consistent with previous studies conducted within Nigeria that reported a higher prevalence of adenotonsillitis in children.[9]

Regarding the distribution of diagnoses in adenotonsillitis cases, adenoidtonsillitis was the most frequent diagnosis, accounting for 82% of the cases. This finding aligns with studies conducted in Nigeria which reported adenoidtonsillitis as the most common diagnosis in cases of tonsillar diseases.[10] Adenoid tonsilar tissue lymphoid hyperplasia and adenoid follicular hyperplasia were identified in 14% and 4% of the cases, respectively. These findings are consistent with the literature, which suggests that lymphoid hyperplasia is a common pathological finding in adenotonsillitis cases.[10]

Gram's reaction and Congo red staining were performed to analyze the microbial presence and amyloid deposition in adenotonsillitis cases. Gram's reaction showed a positive result in 70% of the cases, indicating the presence of Gram-positive bacteria. This finding is supported by studies conducted both within Nigeria, and outside Nigeria, which reported the involvement of bacteria in tonsillar infections.[11,12] Congo red staining, which detects amyloid deposition, was positive in 60% of the cases. The presence of amyloid deposition is consistent with the findings which reported the association of biofilm polysaccharide matrix with chronic tonsillitis.[13]

Furthermore, GMS staining was positive in 18% of the cases, indicating the presence of fungal elements. This finding is in line with studies which reported the involvement of fungi in tonsillar infections.[14]

The study revealed that adenoidtonsillitis was more prevalent in females (22 cases) than males (19 cases). This finding is consistent with a study conducted that reported a higher incidence of tonsillar diseases in females. Adenoid tonsilar tissue lymphoid hyperplasia and adenoid follicular hyperplasia were also observed in both genders, albeit with a smaller number of cases.[15]

The distribution of Gram's reaction, Congo red staining, and GMS staining in different clinical conditions of adenotonsillitis highlighted the variations in microbial presence and amyloid deposition among the different diagnoses. Adenoidtonsillitis showed the highest positive rates for Gram's reaction and Congo Red staining, indicating the involvement of bacteria and amyloid deposition. Adenoid tonsillar tissue lymphoid hyperplasia

and adenoid follicular hyperplasia also exhibited positive results for Gram's reaction but showed varied rates for Congo Red staining and GMS staining. These findings suggest that different clinical conditions of adenotonsillitis may have distinct etiological factors and pathogenic mechanisms.

The results also shed light on the geographical distribution of the cases. All the diagnosed cases of adenotonsillitis, as well as adenoid tonsillar tissue lymphoid hyperplasia and adenoid follicular hyperplasia, were from within the metropolis. this finding suggests that the study population predominantly resided in urban areas. It would be interesting for future studies to explore whether there are any geographical differences in the prevalence and clinical characteristics of adenotonsillitis between urban and rural populations.

Limitations

The sample size was relatively small, with only 50 cases included. This may restrict the generalizability of the findings to the broader population. Moreover, the study was conducted in a specific geographical location, and the results may not be representative of the entire country or other regions with different demographic characteristics.

The retrospective design and the use of formalin-fixed paraffin-embedded biopsy samples, should be considered when interpreting the results. Future research could address these limitations by employing prospective study designs and including additional techniques for biofilm detection and characterization.

CONCLUSION

The results of this study provide valuable insights into the age and gender distribution, diagnoses, and laboratory findings in cases of adenotonsillitis. The findings align with previous studies conducted within Nigeria and support existing literature from international studies. The high prevalence of adenoidtonsillitis, along with the involvement of bacteria and formation of polysaccharide within the biofilm matrix highlights the complex nature of this condition. Future research with larger sample sizes and diverse populations is warranted to further explore the epidemiology, etiology, and pathogenesis of adenotonsillitis. These insights can contribute to the development of effective strategies for diagnosis, treatment, and prevention of adenotonsillitis in Nigeria and globally.

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REFERENCES

1. Galli J, Calò L, Ardito F, Imperiali M, Bassotti E. Adenotonsillitis: Epidemiology, Clinical Presentation, and Treatment. J Int Med Res. 2017;45(4):1512-9.
2. Costerton JW, Stewart PS, Greenberg EP. Bacterial Biofilms: A Common Cause of Persistent Infections. Science. 1999;284(5418):1318-22.
3. Hoa M, Tomovic S, Nistico L, Hall-Stoodley L, Stoodley P, Sachdeva L et al. Identification of Adenoid Biofilms with Middle Ear Pathogens in the Pathogenesis of Chronic Sinusitis. Int J Pediatr Otorhinolaryngol. 2012;76(11):1632-8.
4. Donlan RM. Biofilms: Microbial Life on Surfaces. Emerging Infect Dis. 2002;8(9):881-90.
5. World Medical Association. 2013. Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. Available at: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/. Accessed on 20 June, 2023.
6. Arciola CR, Campoccia D, Montanaro L, Speziale P. Biofilm Formation in Staphylococcus Implant Infections. A Review of Molecular Mechanisms and Implications for Biofilm-Resistant Materials. Biomaterials, 2015;33(26):5967-82.
7. O'Toole GA. Microtiter Dish Biofilm Formation Assay. J Visualized Exp. 2011;(47):e2437.
8. Ramage G, Mowat E, Jones B, Williams C, Lopez-Ribot J. Our Current Understanding of Fungal Biofilms. Crit Rev Microbiol. 2009;35(4):340-55.
9. Onyeagwara NC, Okhakhu AL, Eche VC. Prevalence and management of sore throats and tonsillitis in a boarding school in Benin City. Ann Biomed Sci. 2018;1596-6569.
10. Iliyasu YS, Mohammed AU, Abdulrazak A, Dotiro C, Ibrahim BM, Hamisu A et al. Adenoid and Tonsil Hypertrophy in Zaria, North Western Nigeria: Review of Clinical Presentation and Surgical Outcome: J West Afr Coll Surg. 2022;12(1):23-7.
11. Maduka DU, James OA, Nneamaka E, Nneka GM, Somkene O, Linda A et al. The profile of microorganisms that associate with acute tonsillitis in children and their antibiotics sensitivity pattern in Nigeria: Scientific Reports. 2021;11:20084.
12. Veraluce PC, Leandro AC, Fabiano SM, Edson JMF, Juliana LC, Carla A et al. *Staphylococcus aureus* in tonsils of patients with recurrent tonsillitis:

prevalence, susceptibility profile, and genotypic characterization. Brazilian J Infect Dis. 2019;8-14.
13. Richard AC, Brian TF. Anatomical Evidence of Microbial Biofilms in Tonsillar Tissues A Possible Mechanism to Explain Chronicity: J Am Med Association. 2003;129(6):634-6.
14. Nuutinen J, Kunnas K, Seppä J, Kärjä J, Kolonen S. The mycotic flora of adenoids and antibodies to Candida albicans in children: Arch Otorhinolaryngol. 1986;243(3):194-6.
15. Alyaa A, Emad M. Sex differences in tonsillitis: Dalhousie Med J. 2008;35(1).

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