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Original Research Article

A Comparative Study on Microbiological Profile and Antibiogram of Surface and Core Microflora in Patients with Chronic Tonsillitis

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Abstract

Background: If culture of tonsillar surface is representative of bacteriology of core, then rational therapy could be directed at organisms cultured by surface swab. If not then it should be directed to the core microorganisms. As there is paucity of reports on the microbiological profile and antibiogram of chronic tonsillitis patients in the region of Western Uttar Pradesh, we intended to compare the growth pattern of organisms through surface throat swab culture in preoperative period and tonsil core culture following tonsillectomy.

Methods: The present hospital descriptive cross-sectional study over a period of 12 months among patients (3 years or above) admitted for tonsillectomy for Chronic tonsillitis. The minimum sample size was calculated as 97 considering. During OPD hours or after admission, clinical history was taken and patient-specific and relevant information (age, sex, address and clinical information. Investigations for pre-anaesthesia fitness was done for all patients prior to surgery. The specimen was cultured on 5% sheep blood agar, chocolate agar and in brain heart infusion agar for anaerobic cultivation. Antibiotic susceptibility testing of the organisms was done by Kirby Bauer disk diffusion method (Donald C. Sockett DVM) in Muller Hinton agar. The chi-square test was used to find the difference between dependent (surface isolates vs core isolates) and independent variables (bacterial culture reports).

Results: In present study, the aerobic bacteria in surface and core isolates (n=94) were 88.3% and 94.7%. Staphylococcus aureus were statistically more in proportion in surface isolates as compared to core isolates ($p<0.05$) and Streptococcus pneumoniae were statistically more in proportion in core isolates as compared to surface isolates ($p<0.05$). In present study, the aerobic bacteria in surface and core isolates (n=94) were 17.0% and 13.8%. In present study no statistically significant difference in antibiotic sensitivity for Amikacin, Ciprofloxacin, Cefoperazone & Sulbactam, Ceftriaxone, Azithromycin, Cefepime and Gentamycin among core and surface islolates of β haemolytic streptococci, Staphylococcus aureus, Klebsiella sp, and Streptococcus pneumoniae was observed respectively ($p>0.05$).

Conclusion: Knowing the bacteriology does not assist us cure recurrent tonsillitis because the mechanism of infection activation is unknown. It could, however, be a first step toward determining if bacteria play a role in reactivating recurring infections.

Keywords: Microbiological profile, antibiogram, chronic tonsillitis, surface and core microflora, comparative study

Introduction

Tonsils are subepithelial lymphoid tissue in the oropharynx between the palatoglossal pillar anteriorly and the palatopharyngeal pillar posteriorly. The disease is mainly diagnosed on history and physical examination. Tonsils are in a region where microorganisms are found in ample. Microorganisms penetrate into the tonsillar tissue through the defect in the epithelium and get access to the lymphatic system, which is responsible for all the individual attacks of chronic tonsillitis [1]. Tonsils are important structures of the immune system and tonsillectomy is a very common surgery [2]. Tonsillectomy is indicated in recurrent acute tonsillitis for at least two years with five or more acute attacks per year [3].

Some studies have shown that the bacterial pathogens related to tonsillitis inhabit both the surface and depth of the tonsil tissue [4]. Antimicrobial treatment often fails to eradicate the pathogens and therefore causes recurrences of the tonsillar infection. Swabs from the surface of the tonsil surface are used as a guide in identifying offending organism and proper selection of therapy in acute and recurrent/chronic tonsillitis [5].

Failure to eradicate pathogenic organisms in the core can be due to inappropriate antimicrobial therapy or from inadequate antibiotic penetration in the core, paving way to either persistence of core infection or re-inoculation of the initially sterilized surface. Surgical excision of tonsil is the best modality of treatment for recurrent infection, as pathogens from core of the tonsil remain unidentified and are resistant to the antibiotic therapy based on the tonsillar surface microflora [6].

The purpose of this study is to analyze the difference of microflora in the underlying bacterial pathogens in tonsillar disease of both surface and core region of the palatine tonsils in this geographic area. Some previous studies conclude that the determination of the surface flora alone is not useful in predicting the chronic infectious state [7,8,9]. Thus, comparing the surface and core microflora can provide the otorhinolaryngologist to determine the etiological agent and to initiate treatment successfully and also reducing the quickly emerging impending antibiotic resistance in this group of patients. If culture of tonsillar surface is representative of bacteriology of core, then rational therapy could be directed at organisms cultured by surface swab. If not then it should be directed to the core microorganisms. As there is paucity of reports on the microbiological profile and antibiogram of chronic tonsillitis patients in the region of Western Uttar Pradesh, we intended to compare the growth pattern of organisms through surface throat swab culture in preoperative period and tonsil core culture following tonsillectomy. The expected outcome will be helpful for the surveillance and antimicrobial susceptibility generally and in particular of tonsillar material. This further helps in early detection of emerging resistance trends and adjustment and usage of appropriate therapeutic interventions.

Materials and Methods

Study setting and Design

The present hospital descriptive cross-sectional study was carried in the Department of Otorhinolaryngology, Government Institute of Medical Sciences, Greater Noida, Uttar Pradesh, India over a

period of 12 months from September 2019 to October 2020, after obtaining ethical approval from Institutional Ethics Committee (IEC/IRB No. AIHFEC/04/023; Ahalia International Foundation Ethics Committee, Kozhippara).

Study subjects and sample size

The present study included patients (3 years or above) admitted for tonsillectomy for Chronic tonsillitis (described as more than seven episodes of acute attacks in one year, more than five acute attacks for two years, more than three attacks for three consecutive year, or two weeks of school or work lost in one year due to tonsillitis) and causing obstructive symptoms like difficulty in swallowing as study subjects [10]. The minimum sample size was calculated as 97 considering the prevalence of growth on surface swabs as 62% and taking absolute precision as 10% [11]. Prior to enrolling of subjects into the study, written informed consent was obtained either from patient or relatives after explaining in detail regarding the purpose of study, and consecutive sampling method was used to enrol the study subjects, so a total of 150 patients were enrolled in the study during defined study duration. Patients on antibiotics within the past 2 weeks, patients undergoing tonsillectomy for reasons other than chronic tonsillitis, immunocompromised patient and patients with chronic tonsillitis but medically unfit for surgery were excluded from the study.

Data and Culture Sample collection

During OPD hours or after admission, clinical history was taken and patient-specific and relevant information (age, sex, address and clinical information, including chief complaints and duration of symptoms) was collected in a structured data collection schedule through interviews. Following this a detailed Otorhinolaryngological and general physical examination was done. Investigations for pre-anaesthesia fitness was done for all patients prior to surgery. Tonsillar surface swab was taken for all cases of chronic tonsillitis posted for tonsillectomy before commencement of antibiotic therapy. Swab were collected by rotating a sterile cotton swab over the surface of the tonsils without touching other parts of the oropharynx. It was placed in sterile test tube and transported to microbiology lab. All patients undergone tonsillectomy by dissection and snare method. Excised tonsils were placed in normal saline. Tonsil tissue was cut with sterile blade and swab were taken from inner surface which was inoculated to culture media incubated for a period of 24 – 48 hours. These were then transferred to the bacteriology laboratory. The specimen was cultured on 5% sheep blood agar, chocolate agar and in brain heart infusion agar for anaerobic cultivation. The plates were incubated at 37°C in the presence of 5-10% CO_2 for 24 to 48 hours. Subculture from Robertson's cooked meat medium has been also done. Colony identification was accomplished using the standard technique. Biochemical confirmations were also done according to the standard bacteriological methods. Growths of β haemolytic streptococci, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus spp, Klebsiella spp, and Proteus spp on any of the media were identified by standard methods. β hemolytic streptococcal (BHS) colonies could not be classified further into their Lancefield groups because of non-availability of this facility. Antibiotics such as amikacin, ciprofloxacin, cefoperazone & sulbactam, ceftriaxone, azithromycin, cefepime and gentamycin were used in the study for sensitivity. Antibiotic susceptibility testing of the organisms was done by Kirby Bauer disk diffusion method (Donald C. Sockett DVM) in Muller Hinton agar. The plates were read after overnight incubation at 37^0c, by measuring the zone of inhibition around the antibiotic discs and reference tables were used to determine if the bacteria are sensitive (S), intermediate (I) or resistant (R) to the antimicrobial

drugs as per CLSI (Clinical Laboratory Standards Institute) guidelines.

Statistical analysis

The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 28. Results were analysed with baseline demographic and clinical, culture reports of isolated of each group (surface vs core) of study patients. Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD. Normality of data were tested by Kolmogorov-Smirnov test. If the normality was rejected, then non parametric test was used. The chi-square test was used to find the difference between dependent (surface isolates vs core isolates) and independent variables (bacterial culture reports). All tests were performed at a 5% level of significance; thus, an association was significant if the p value was less than 0.05.

Results

In the present study, one third of patients were males (34.7%) and two third of patients were female (65.3%). Half of the patients were of 6-12 years of age (55.3%) and one third were between 13-18 years (31.4%). The indication for tonsillectomy among patients was recurrent tonsillitis (94.7%), followed by tonsillar size (25.3%) and general ill health (16.7%). On clinical examination 70.7% of patients had palpable jugulodigastric nodes and squeeze test was positive among 22.7% of patients. The bacterial culture of tonsillar together core and surface isolates showed that overall 32.7% of isolates were mono-bacterial, 30.0% of isolates were poly-bacterial and remaining 37.3% of isolates were sterile (Table 1).

Table 1: Baseline characteristics of the patients (N=150)

Baseline characteristics	Number	%
Gender		
Male	52	34.7
Female	98	65.3
Age group (in years)		
4-5	6	4.0
6-12	83	55.3
13-18	47	31.4
>18	14	9.3
Indication for tonsillectomy*		
Recurrent tonsillitis	142	94.7
Tonsillar size	38	25.3
General ill health	25	16.7
Palpable jugulodigastric nodes		
Yes	106	70.7
No	44	29.3
Squeeze test		
Positive	34	22.7
Negative	116	77.3
Bacterial involvement in isolates		
Mono-bacterial	49	32.7
Poly-bacterial	45	30.0
Sterile	56	37.3

*Multiple responses

In present study, the aerobic bacteria in surface and core isolates (n=94) were 88.3% and 94.7%. The proportion of β haemolytic streptococci, Staphylococcus aureus, Klebsiella sp, Streptococcus pneumoniae, Proteus sp and Enterococcus in surface isolates were 39.4%, 22.3%, 19.1%, 12.8%, 6.4% and 3.2% respectively. The proportion of β haemolytic streptococci, Staphylococcus aureus, Klebsiella sp, Streptococcus pneumoniae, Proteus sp and Enterococcus in core isolates were 33.0%, 9.6%, 22.3%, 25.5%, 10.6% and 5.3% respectively. Staphylococcus aureus were statistically more in proportion in surface isolates as compared to core isolates ($p<0.05$) and Streptococcus pneumoniae were statistically more in proportion in core isolates as compared to surface isolates ($p<0.05$). In present study, the aerobic bacteria in surface and core isolates (n=94) were 17.0% and 13.8%. The proportion of Bacteriodes sp, Fusobacterium sp and Peptostreptococcus sp in surface isolates were 8.5%, 7.4% and 4.3% respectively, whereas their proportions in core isolates were 6.4%, 5.3% and 5.3% respectively (Table 2).

Table 2: Bacterial profile in the tonsillar surface and core isolates of the patients (N=94).

Type of isolate*	Surface	Core	P value
Aerobic bacteria	83 (88.3)	89 (94.7)	>0.05
β haemolytic streptococci	37 (39.4)	31 (33.0)	>0.05
Staphylococcus aureus	21 (22.3)	9 (9.6)	<0.05#
Klebsiella sp	18 (19.1)	21 (22.3)	>0.05
Streptococcus pneumoniae	12 (12.8)	24 (25.5)	<0.05#
Proteus sp	6 (6.4)	10 (10.6)	>0.05
Enterococcus sp	3 (3.2)	5 (5.3)	>0.05
Anaerobic bacteria	16 (17.0)	13 (13.8)	>0.05
Bacteriodes sp	8 (8.5)	6 (6.4)	>0.05
Fusobacterium sp	7 (7.4)	5 (5.3)	>0.05
Peptostreptococcus sp	4 (4.3)	5 (5.3)	>0.05

*Multiple responses, #Statiscally Significant

Antibiotics sensitivity for Amikacin, Ciprofloxacin, Cefoperazone & Sulbactam, Ceftriaxone, Azithromycin, Cefepime and Gentamycin among surface isolates of Streptococcus pneumoniae were 81.9%, 77.7%, 80.9%, 67.0%, 57.4%, 56.4% and 74.5% respectively, whereas sensitivity of those antibiotics for core isolates of Streptococcus pneumoniae were 84.0%, 79.8%, 86.2%, 68.1%, 55.3%, 53.2% and 76.6% respectively (Figure 1).

Antibiotics sensitivity for Amikacin, Ciprofloxacin, Cefoperazone & Sulbactam, Ceftriaxone, Azithromycin, Cefepime and Gentamycin among surface isolates of β haemolytic streptococci were 77.7%, 72.3%, 83.0%, 56.4%, 57.4%, 54.3% and 72.3 % respectively, whereas sensitivity of those antibiotics for core isolates of β haemolytic streptococci were 79.8%, 75.5%, 89.4%, 60.6%, 58.5%, 58.5% and 79.8% respectively (Figure 1).

Antibiotics sensitivity for Amikacin, Ciprofloxacin, Cefoperazone & Sulbactam, Ceftriaxone, Azithromycin, Cefepime and Gentamycin among surface isolates of Staphylococcus aureus were 79.8%, 78.7%, 86.2%, 60.6%, 59.6%, 56.4% and 75.5% respectively, whereas sensitivity of those antibiotics for core isolates of Staphylococcus aureus were 80.9%, 75.5%, 89.4%, 57.4%, 54.3%, 58.5% and 80.9% respectively (Figure 1).

Antibiotics sensitivity for Amikacin, Ciprofloxacin, Cefoperazone & Sulbactam, Ceftriaxone, Azithromycin, Cefepime and Gentamycin among surface isolates of Klebsiella sp. were 70.2%, 76.6%, 72.3%, 71.3%, 52.1%, 56.4% and 78.7% respectively, whereas sensitivity of those antibiotics for core isolates of Klebsiella sp. were 77.7%, 79.8%, 76.6%, 75.5%, 53.2%, 56.4% and 80.9% respectively (Figure 1).

In present study no statistically significant difference in antibiotic sensitivity for Amikacin, Ciprofloxacin, Cefoperazone & Sulbactam, Ceftriaxone, Azithromycin, Cefepime and Gentamycin among core and surface islolates of β haemolytic streptococci, Staphylococcus aureus, Klebsiella sp, and Streptococcus pneumoniae was observed respectively ($p>0.05$)

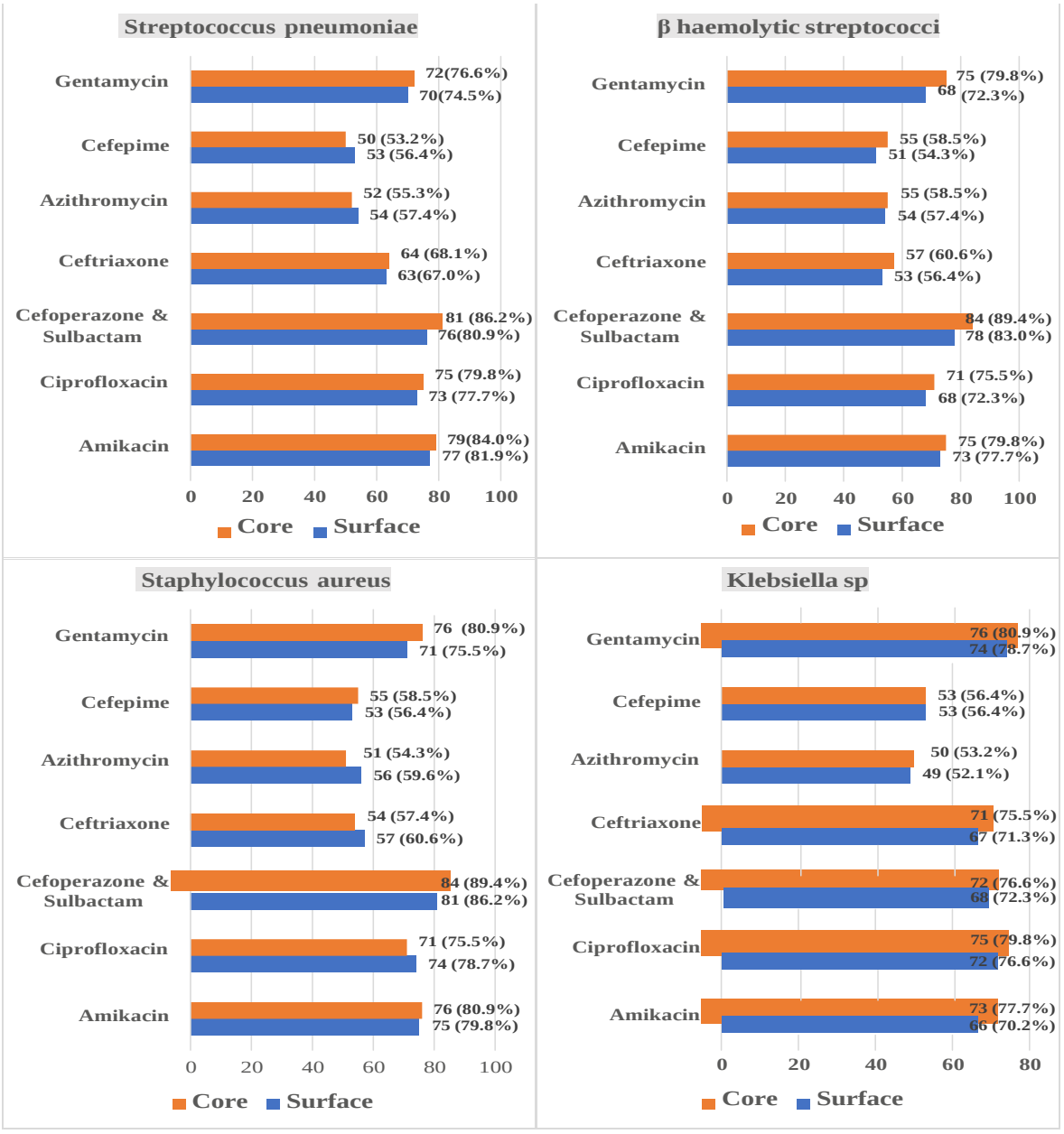

Figure 1: Antibiogram of the tonsillar and core isolates of the patients (N=94).

Discussion

Tonsillectomy is indicated in chronic tonsillitis, described as more than seven episodes of acute attacks in one year, more

than five acute attacks for two years, more than three attacks for three consecutive years, or two weeks of school or work lost in one year due to tonsillitis. The procedure may be associated with transient bacteremia in some cases which may or may not be associated with any enhanced post-operative morbidity [10]. Superficial tonsillar swabs are often used as a guide in identifying the offending organism and the proper selection of therapy in acute and recurrent tonsillitis [5]. Normal bacterial flora present in the oral cavity and oropharynx may be cultured in most cases and antibiotic therapy is instituted based on the results obtained from the cultures grown by the throat swab.

In present study, the most common extracted isolate was β haemolytic streptococci and Staphylococcus aureus. Unlike nasal mucus where staphylococcus isolates can exist as the normal flora, it does not apply to tonsil's tissue and mucus, therefore the presence of staphylococcus isolate is an indication of pathogen existence. Regarding the prevalence of staphylococcus aureus, the results of the current study are in accordance of outcomes of similar studies. However, Klebsiella sp, and Streptococcus pneumoniae prevalence were higher [12,13]. In Chronic tonsillitis, which is diagnosed mainly by history and clinical examination, the most frequent bacteria are Hemophilus influenzae, followed by Staphylococcus aureus and Streptococcus pyogenes. A high tissue concentration of these bacteria correlates with clinical parameters of infection and hyperplasia of the tonsils [11].

Most of the research work on the microbiological study of chronic tonsillitis is aimed at identifying the aerobic organisms. The role of anaerobes in chronic tonsillitis is rarely studied. Anaerobes are normally commensal in the oropharynx, so cultures taken from the surface may be misleading. In few studies, the role of both aerobes and anaerobes were included. Anaerobes though not studied on a regular basis in cases of chronic tonsillitis, are recognized causative organisms in infection and recurrence of the disease [14].

Because the tonsillar surface is contaminated with oropharyngeal secretions, it generally shows normal flora of the oropharynx. Oropharyngeal flora contains aerobic and anaerobic bacteria, including alpha-hemolytic and nonhemolytic Streptococci, coagulase negative Staphylococci, Neisseriae, Corynebacteria, Actinomyces, Leptotrichiae and Fusobacterium species. Bacterial agents such as Group A Beta hemolytic Streptococci, Staphylococcus aureus, Hemophilus influenza, Streptococcus pneumonia, Corynebacterium diptheriae and Neisseria which may be disrupted by frequent use of broad-spectrum antimicrobials, by inhibiting sensitive organisms and allowing overgrowth of the resistant ones. This may cause serious infection by the normal commensals [15]. Despite the fact that tonsillitis is so common, consensus seems to be lacking as to the main causative organisms and its differences in children and adults [16].

There is strong anatomical evidence for the presence of bacterial biofilms in chronically diseased tonsils. Because sessile bacteria within biofilms are resistant to host defenses and antibiotics, bacterial biofilms within tonsils may explain the chronicity and recurrent nature of some forms of tonsillitis [17,18]. The probable causes of recurrence in chronic tonsillitis are; penicillin resistance due to the variations of the oropharyngeal flora, nonspecific antibiotic treatments, reinfection from the environment, suppression of the antibody response due to the previous inappropriate antibiotic therapies [19]. In few studies, organisms isolated from the tonsil surface did not always correspond with the organisms isolated from the deep tissue specimens. While the surface cultures commonly showed entirely normal flora, the tonsil

core cultures yielded pathogenic microorganisms [20].

In present study most isolates had a higher antibiotic resistance which is likely due to inappropriate prescription and consequently inaccurate consumption of antibiotics in recent years. On the other hand, resistance was also observed in very new antibiotics and if this trend continues, they will have the same fate as older ones. The next problem was the high resistance to penicillin, the most frequent medicine prescribed for tonsillitis treatment, in addition that this is an alarm for clinical specialists, it notifies the need to change the selected medicine for curing recurrent and chronic tonsillitis. The cultivated bacteria were not meaningfully different regarding age and indication of surgery. Additionally, since isolates reveal a high resistance to penicillin if we tend to decrease the size of tonsils without surgery, for any reason, the antibiotic selection matters. [21] Therefore, based on present findings penicillin is not a suitable choice. Microorganisms other than Group A Beta Hemolytic Streptococcus (GABHS) may be the cause of chronic tonsillitis. Especially in recent years the domination of the beta-lactamase producing bacteria (BLPB) such as Staphylococcus aureus and Hemophilus influenzae in microflora cause penicillin resistance. Several researchers have claimed that the failure of antibiotherapy may be due to the underestimation of the resistant microorganisms [19].

Conclusion

Knowing the bacteriology does not assist us cure recurrent tonsillitis because the mechanism of infection activation is unknown. It could, however, be a first step toward determining if bacteria play a role in reactivating recurring infections. There is no link between bacteriology and recurring illnesses, according to prior and current research. As a result, precise identification of bacterial isolates in tonsillitis, as well as their antibiotic sensitivity pattern, could change chronic tonsillitis care.

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