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Article

Prevalence of parallel infection of dental caries and tonsillitis among some Iraqi children

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Abstract: Aims and Objectives: This study aims to investigate the role of dental caries-causing bacteria in increasing acute or chronic tonsillitis among Iraqi children. Materials and Methods: 120 patients were part of the study and were divided into 6 groups; each group had 20 patients aged 6–12. Swab samples were obtained from dental plaque and tonsils to identify the bacterial flora. Identification methods included metabolic capability, microscopic reading, and biochemical reaction. Results: The results showed that *Streptococcus pneumonia* was the most common Gram-positive bacterial species isolated from three different groups and control rate (32.9 %). Also, *Moraxella catarrhalis, Granulicatella elegant and Streptococcus parasanguinis appeared in both tonsillitis and dental caries.* Conclusion: An interesting association between dental caries and tonsillitis in children aged 6 to 12 appeared. Most Gram-positive and Gram-negative bacterial isolates were multi-drug resistant to antimicrobial agents.

Keywords: parallel infection, dental caries, tonsillitis, Iraqi children.

Introduction

Dental caries is a biofilm-mediated, sugar-driven, multi-factorial, dynamic illness that results in the Dental phasic remineralization and demineralization of dental hard tissue[1]. In severe conditions, it initially affects the upper front teeth of children and eventually spreads to the primary first molars[2]. Tonsillitis is the inflammation of the pharyngeal tonsils and typically affects children. Tonsillitis produced by Streptococcus species is uncommon in children younger than 2, but viral tonsillitis is more prevalent in children younger than 5. [3] It is contagious and can be spread through sharing food, beverages, and utensils with infected individuals. Tonsillitis affects children's health and quality of life and generates significant morbidity and school absences. [4] The adenoids (nasopharyngeal tonsil), tubal tonsil, and lingual tonsil are all parts of Waldeyer's ring, which comprises lymphatic tissue, including the tonsils[5]. No previous studies were conducted in Iraq and the Middle East to assess the relationship between dental caries and tonsillitis for ages 6-12 years. Therefore, this study is critical because it provides an incredible view of the relationship between tooth decay and tonsillitis. Antibiotic resistance is a genetic mutation that confers acquired resistance to antibiotics. In contrast, antibiotic tolerance is a transient and non-heritable physiological state of biofilm cell populations [6]. Antibiotic resistance is a global health concern that requires immediate attention since effective treatment of infectious diseases is crucial to human and animal health administration[7]. Antibiotic resistance results in higher resource consumption, clinical or economic costs, and the use of broad-spectrum antibiotics, as well as

increased morbidity and mortality [8]. To investigate the role of dental caries-causing bacteria in increasing acute or chronic tonsillitis among Iraqi children.

Material and methods

Obtaining of the samples

The study was conducted among 120 children aged 6-12 years; 120 oral samples were collected from different mouth sites (teeth, tonsils, and throat) from 120 patients who visited the dental clinical unit and Otolaryngologic department in Hospital of Baghdad Medical City and Ibn Al Baladi Hospital for children and women of, whose were of both sexes and ages. These samples were divided into 6 groups: (1 dental caries, 2 tonsillitis, 3 tonsillitis and dental caries, 4 tonsillectomy, 5- 5 tonsillectomy and dental caries, and 6-control).

Sample processing, standard plate counting and identification of the bacterial isolates

Amie's transport medium was used to transport the samples; then, specimens were transported to the laboratory and cultured on Blood agar and MacConkey agar. Moreover, all plates were incubated under aerobic conditions at 37 C for (24– 48) hours, and the specimen was cultured on chocolate agar under 5% CO2 in a candle jar for isolation and identification. All colonies from primary cultures were purified by subculture on different media and then identified. Species were identified according to the morphological features on culture media, microscopic examination, biochemical tests and Vitek 2 system. Ethical approval was taken for conducting this study.

Antibacterial sensitivity test

antibiotic susceptibility test was directed for all 100 tested isolates in the current study by using the Kirby-Bauer method, which relied on measuring the diameter of the inhibition zone and comparing it with the Clinical and Laboratory Standards Institute (CLSI, 2020) as susceptible, intermediate and resistant towards 15 antimicrobial agents that categorized into eight classes: penicillins, cephalosporins, carbapenems, macrolides, sulphonamide dihydrofolate reductase, aminoglycosides, chloramphenicol and fluoroquinolones. It has been found that there is a noticeable variation in resistance levels among isolates, especially the same type of species isolated from different groups.

Results

Isolation of bacteria

Identifying the bacterial isolates responsible for infections remains an essential step for the choice of antimicrobial therapy and disease follow-up and treatment. Thus, the current study was interested in the bacterial isolates from different isolation sites selected according to the type of study design listed in Table (1-1); 110 bacterial isolates were collected from different isolation sites during the sample collection period. Bacterial isolates were preliminarily cultured on MacConkey agar, blood agar, and selective and differential media in aerobic and microaerophilic conditions, followed by other differential diagnostic tests. One hundred and ten bacterial isolates represented by different bacterial species appeared exclusively in the tested groups and control as follows: 18(16.4), 14(12.7), 13(11.7), 12(10.9), 12(10.9), 10(9.1), 7(6.4), 7(6.4) from isolation sites as listed in table 1-1.

Tested groups	Bacterial Isolation site	Total of bacterial isolates
G1=Dental caries patients	**Dental caries**	**18(16.4)**
G2=Tonsillitis patients	**Tonsils**	**17(15.5)**
G3a=Tonsillitis AND G3B=dental caries patients	**Dental caries** **And Tonsils**	**14(12.7)** **13(11.7)**
G4a=Tonsillectomy & G4b= dental caries	**Dental caries** **And Tonsils position**	**12(10.9)** **12(10.9)**
G5=Tonsillectomy patients	**Tonsils position**	**10(9.1)**
Negative for all (control)	**Noninfected Tonsils and teeth or gum**	**7(6.4)** **7(6.4)**
Total		**110(100)**

Tested groups	*Kokyria kristinae*	*Granulicatella*	*Gemella morbil-*	*Staphylococcus*	*Streptococcus*	*Strep. Pyogenes*	*Streptococcus*	*Streptococcus*	*Streptococcus*	*Micrococcus*	*Moraxella*	TOTAL

*** G1= Dental caries patients, G2=Tonsillitis patients, G3a=Tonsillitis AND G3B=dental caries patients, G4a=Tonsillectomy & G4b= dental caries, G5=Tonsillectomy patients, C= control**

Table 1-1. Number and percentage Distribution of bacterial isolates according to their isolation site.

Distributions of bacterial isolates

Many genera of Gram-positive and Gram-negative bacteria inhabit the oral cavity, especially from the isolation sites included in this study. Table (1-2) shows the distribution of all bacterial isolates. The results showed that Streptococcus pneumonia was the most common Gram-positive bacterial species isolated from three different groups and control in rate 36 (32.9 %), distributed as follows 9 (8.2%), 12 (10.9%), 5 (4.5%) and 10 (9.1%) isolated from G2 (Tonsillitis patients), G4a (Tonsillectomy), G5 (Tonsillectomy patients) and control respectively. It should be noted that this type of bacteria was not isolated from any samples of dental caries but was isolated only from infected and noninfected tonsils and from tonsil positions in the G4a and G5 groups, as well as from normal tonsils as in the control group. S. pneumonia was not isolated from dental caries because it tends to stick to the tonsil tissue.

Microbiological study showed the presence of Moraxella catarrhalis, Granulicatella elagans and Streptococcus parasanguinis in both tonsillitis and dental caries. On the other hand, Streptococcus mitis was isolated at a rate (of 5.5 %) from dental caries only. The distribution of Staphylococcus aureus was found to be 1.8%. In comparison, Streptococcus pyogenes was isolated from tonsillitis at a rate (of 1.8%), but Gemella morbillorum was isolated at a rate (of 1.8%) from dental caries. Kocuria kristinae, a natural skin and mucosal flora associated with many mammals, was isolated from tonsillectomy patients and control groups at a rate of 5.5%.

	G1=Dental caries patients	**0**	**0**	**2(1.8%)**	**0**	**4(3.6%)**	**0**	**0**	**0**	**0**	**0**	**12(10.9%)**	**18**
	G2=Tonsillitis patients	**0**	**0**	**0**	**2(1.8%)**	**0**	**2(1.8%)**	**3(2.7%)**	**2(1.8%)**	**9(8.2%)**	**0**	**0**	**17**
	G3a=Tonsillitis	**0**	**1(0.9%)**	**0**	**0**	**1(0.9%)**	**0**	**2(1.8%)**	**0**	**0**	**1(0.9%)**	**8(7.3%)**	**14**
	G3b= dental caries patients	**0**	**1(0.9%)**	**0**	**0**	**1(0.9%)**	**0**	**2(1.8%)**	**0**	**0**	**1(0.9%)**	**7(6.4%)**	**13**
	G4a=Tonsillectomy	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12(10.9%)**	**0**	**0**	**12**
	G4b= dental caries	**0**	**5(4.5%)**	**0**	**0**	**0**	**0**	**5(4.5%)**	**0**	**0**	**0**	**0**	**12**
	G5=Tonsillectomy patients	**2(1.8%)**	**0**	**0**	**0**	**0**	**0**	**6(5.5%)**	**0**	**5(4.5%)**	**0**	**0**	**10**
	Negative for all (control)	**4(3.6%)**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**10(9.1%)**	**0**	**0**	**14**
	Total	**6**	**7**	**2**	**2**	**6**	**2**	**18**	**2**	**36**	**2**	**27**	**110**
	%	**5.5**	**6.4**	**1.8**	**1.8**	**5.5**	**1.8**	**16.4**	**1.8**	**32.7**	**2.7**	**24.5**	**100**

Table 1-2. Number and percentage of distribution of bacterial isolate among the tested group.

3. Results of the Antibacterial sensitivity test

The current study showed that 33 out of 36 S. pneumoniae isolates were resistant to penicillin and erythromycin, and 31 of 36 isolates were resistant to trimethoprim/sulphamethoxazole. In comparison, only 22 of 36 isolates were resistant to ampicillin. Other isolates resisted the range of 6-16 antimicrobial agents. In addition, it was found that these isolates were susceptible (100%) to the following antimicrobial agents: cefepime, imipenem and tigecycline; all these results are shown in Figure 1. In contrast, 24 out of 27 M. catarrhalis isolates were resistant to trimethoprim/sulphamethoxazole, and 14 of 27 isolates were resistant to amoxicillin.

In comparison, just 9 of 27 isolates were resistant to cefixime; the resistance of other isolates ranged from 6-8 antimicrobial agents. In addition, it was found that these isolates were susceptible (100%) to the following antimicrobial agents: cefepime, ceftriaxone, imipenem, erythromycin, tigecycline, ofloxacin, moxifloxacin, and ciprofloxacin, All these results are shown in figure 2. The results of the current study showed that 17 out of 18 isolates of Streptococcus parasanguinis were resistant to penicillin, 16 of 18 isolates were resistant to trimethoprim/sulphamethoxazole, 14 of 18 isolates were resistant to ampicillin, 12 of 18 isolates were resistant to cefotaxime, 11 of 18 isolates were resistant to both of amoxicillin and cloxacillin; In comparison, only 8 of 18 isolates were resistant to ceftriaxone, and 3 of 18 isolates were resistant to erythromycin. In addition, it was found that all these isolates were sensitive (100%) to the following antimicrobial agents: cefepime, cefixime, imipenem, tigecycline, ofloxacin, moxifloxacin, and ciprofloxacin. All these results are shown in Figure 3.

Also, in a study performed, out of 7 (100%) of Granulicatella elegant isolates were resistant to ampicillin, 6 of 7 isolates were resistant to penicillin, 5 of 7 isolates were resistant to cefotaxime, ceftriaxone and erythromycin, and only 2 of 7 isolates were resistant to cloxacillin; In contrast, just 1 isolate was resistant to amoxicillin-clavulanic acid. In addition, it was found that these isolates were susceptible (100%) to the following antimicrobials: cefepime, cefixime, imipenem, tigecycline, trimethoprim/sulphamethoxazole, ofloxacin, moxifloxacin, and ciprofloxacin, All these results are shown in figure 4 On the other hand, S. mitis isolates (100%) were resistant to both of amoxicillin/clavulanic acid and penicillin, 4 of 6 isolates were resistant to ampicillin, cefotaxime and ceftriaxone, while just 3 of 6 isolates were resistant to cloxacillin. In addition, it was found that these isolates were susceptible (100%) to the following antimicrobial agents: cefepime, cefixime, imipenem, erythromycin, tigecycline, trimethoprim/sulphamethoxazole, ofloxacin, moxifloxacin, and ciprofloxacin, All these results are shown in figure 5.

In the present study, 6 Kokyria kristinae isolates (100%) were resistant to erythromycin, 2 of 6 isolates were resistant to cloxacillin, ampicillin and trimethoprim/sulphamethoxazole, while just 1 isolate was resistant to amoxicillin/clavulonic acid. In addition, it was found that all these isolates were sensitive (100%) to the following antimicrobial agents: penicillin, cefepime, cefotaxime, ceftriaxone, cefixime, imipenem, tigecycline, ofloxacin, moxifloxacin and ciprofloxacin, All these results are shown in figure 6.

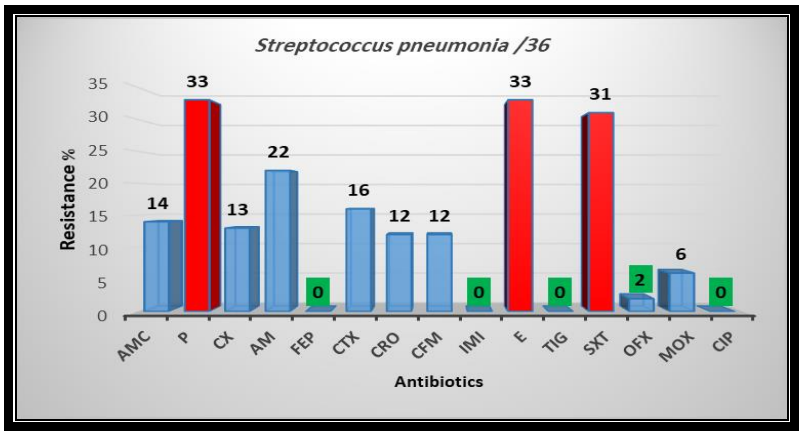

Figure 1. Antibiotic resistance profile of *Streptococcus pneumoniae* isolated from control and all tested groups.

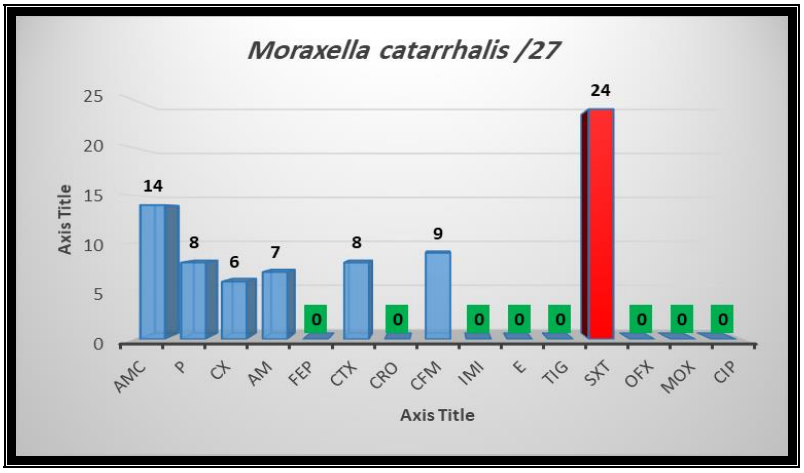

Figure 2. Antibiotic-resistant profile for all *Moraxella catarrhalis* isolates isolated from all tested groups.

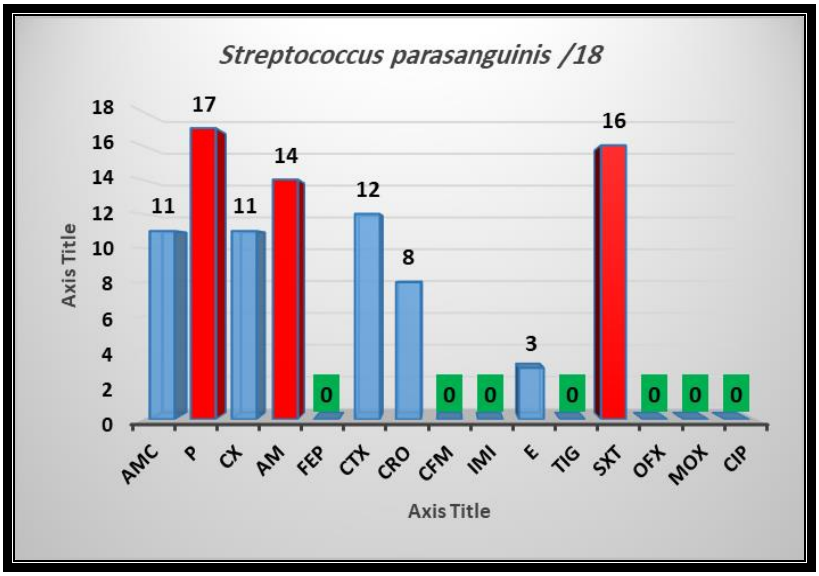

Figure 3. Antibiotic-resistant profile for all *Streptococcus parasanguinis* isolates isolated from all tested groups.

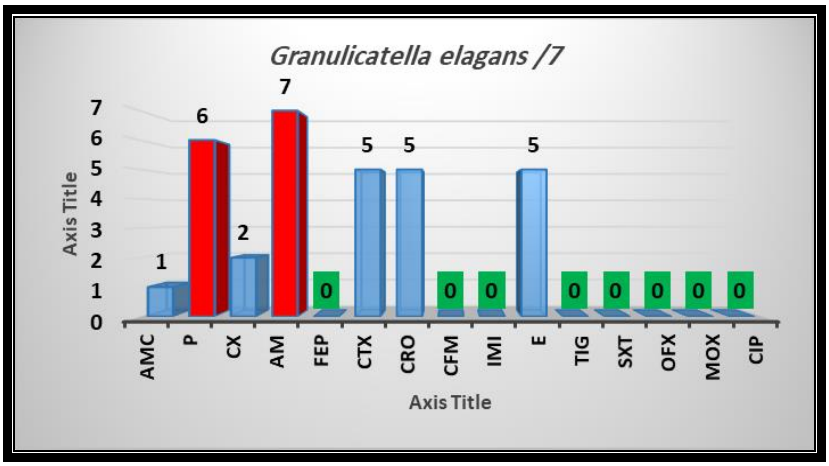

Figure 4. Antibiotic-resistant profile for all *Granulicatella elagans* isolates isolated from all tested groups.

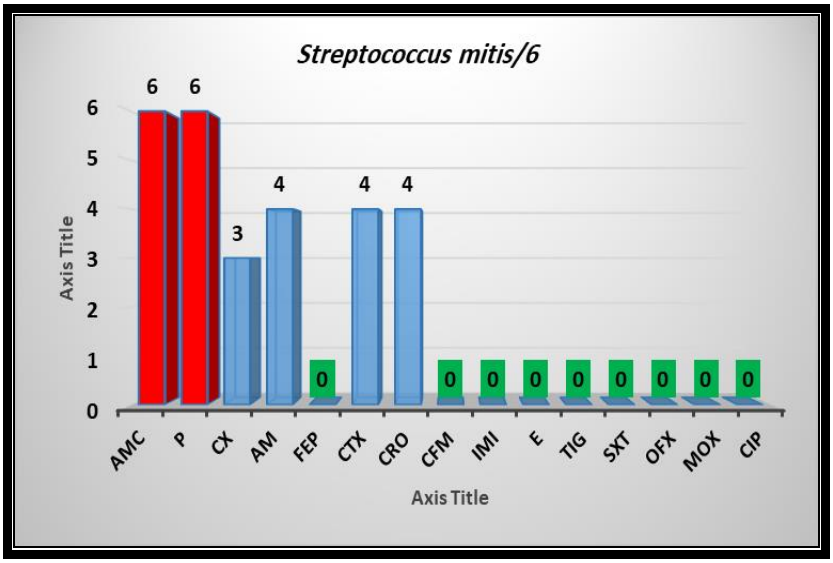

Figure 5. Antibiotic-resistant profile for all *Streptococcus mitis* isolates isolated from all tested groups.

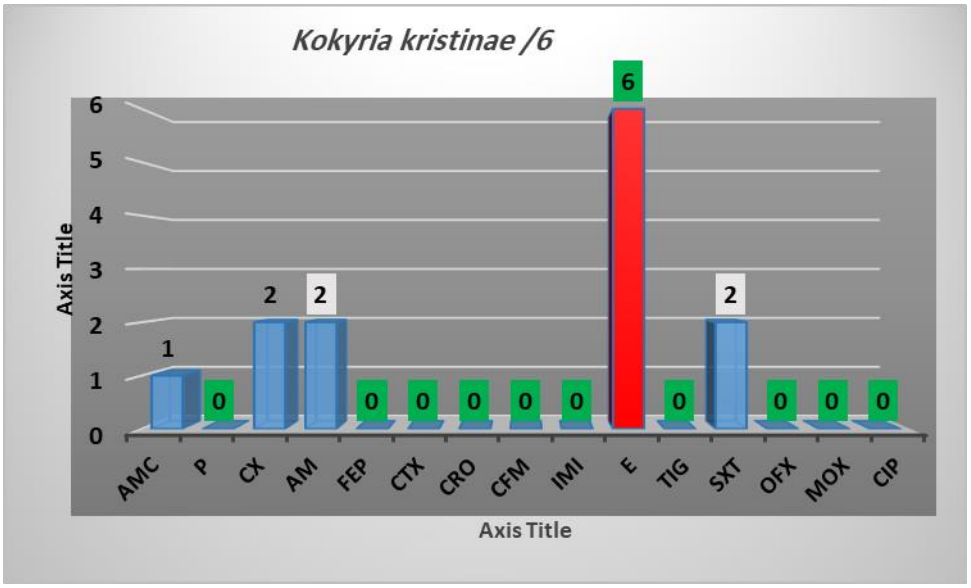

Figure 6. Antibiotic-resistant profile for all *Kokyria Kristina* isolates isolated from all tested groups.

Discussion

The human oral cavity comprises several habitats, including teeth, gingival sulcus, tongue, cheeks, hard and soft palates, and tonsils, which bacteria colonize. The oral microbiome comprises over 700 prevalent taxa at the species level, with distinct subsets predominating at different habitats [9]. The bacterial types isolated from all tested group specimens have been extensively characterized depending on the diagnostic patterns of aerobic and facultative anaerobic bacteria employing standard bacteriological techniques [10]. Al-Oebady et al. conducted a study in Iraq and found that many types of bacteria were isolated from oral cavit[ies11] Anaerobic Gram-negative bacteria and Streptococci are the main types of human mouth's normal flora [12]. The findings agree with Al-Gebori, who also reported a predominant incidence of Streptococci over Staphylococcus [13] while disagreeing with Cockerill et al., who observed a primary occurrence of Staphylococcus over Streptococci [14]. Microbiological study showed that in the presence of Moraxella catarrhalis, Granulicatella elagans and Streptococcus parasanguinis in both tonsillitis and dental caries, there seems to be an association between dental caries and tonsillitis in children aged 6-12 years old. It may be accepted that the infection passes much more frequently from the teeth to the tonsils than in the opposite direction. This result agreed with [15,16], who revealed a positive association between dental caries and tonsillitis. Approximately 10% of adult dental plaque is colonized by G. elegans. [17] The interface between tooth and tissue is a typical entrance point for germs into the body [18]. Streptococcus parasanguinis is a species of the viridian streptococci, which comprise most of the human oral microbial environment. S. parasanguinis is among the earliest colonists of the tooth surface in its primary niche. Moraxella catarrhalis is an exclusively human commensal and mucosal pathogen [19]. It is a common commensal organism of the upper respiratory tract, particularly in children. Streptococcus mitis is a species of the viridian streptococci family; they are widespread in the human mouth and usually are not harmful. However, they can sometimes cause bacterial endocarditis when they colonize oral hard tissues, like the teeth and mucous membranes in the mouth. In the present study, Streptococcus pyogenes was isolated at a rate (of 1.8%) Hamda et al. reported

showing the prevalence of bacterial isolates; Streptococcus pyogenes was the most frequent bacterial isolate from children with tonsillitis and its percentage (55%) [20]. On the other hand, Gemella morbillorum was isolated at a rate (of 1.8%) from dental caries, which is only a bacterium belonging to the family of the Gemella species. These microorganisms are Gram-positive cocci and facultative anaerobes, which—like other human commensal bacteria—are opportunistic pathogens and may cause severe local and systemic infection, mainly in immunosuppressed patients [21]. Kocuria kristinae, a natural skin and mucosal flora associated with many mammals, was isolated from tonsillectomy patients and control groups at a rate of 5.5%. It behaves as an opportunistic pathogen. The genus Kocuria has 18 species, five of which are pathogenic [22]. As previously mentioned, the organism has been found in various bodily fluids, including sputum, blood, bile, synovial fluid, urine, and throat swabs [23]. Antimicrobial Resistance (AMR) is an ancient, natural, widespread phenomenon that precedes humanity [24]. Different genetic and biochemical pathways involving multiple facets of bacterial cell function may be the leading cause of AMR [25]. Resistance is the inherited ability of a bacterium to grow when exposed to high concentrations of antibiotics [26]. During recent years, it has become increasingly clear that the future of antibiotic therapy will rely on the development of resistance not only among pathogens but also among commensal species that can serve as reservoirs for resistance genes [27].

The results were consistent with the study of Iliyasu et al., who found a high prevalence of penicillin resistance. Furthermore, cefotaxime and ceftriaxone have excellent activity against S. pneumoniae, possibly attributable to their scarcity and higher cost than penicillin [28]. Share et al. found intermediate and fully resistant rates for erythromycin and penicillin to be 17.5% and 26.3%, respectively. The corresponding values for ceftriaxone and cefotaxime were 1.8% and 3.5%, while the resistance rates to clindamycin, erythromycin, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, and were 17.5%,59.6%, 17.5%, 38.6%, 24.6% respectively[29].

The results correspond with the study of Hsu et al., who found no resistance against moxifloxacin, ciprofloxacin and erythromycin with high resistance towards trimethoprim/sulphamethoxazole [30]. On the other hand, Monzer et al. found M. catarrhalis susceptibility was 100% to almost all used antibiotics, such as amoxicillin-clavulanic acid, cefotaxime, cefixime, tetracycline, minocycline, erythromycin, and chloramphenicol, with high resistance rates towards ampicillin (92.6%), trimethoprim-sulfamethoxazole (14.7%), nalidixic acid (14.7%), and ciprofloxacin (2.9%) [31].

According to the study of Dhotre et al., S. parasanguinis exhibited high susceptibility levels to penicillin, ampicillin, ceftriaxone and vancomycin, with moderate resistance to cefepime cefotaxime, erythromycin and azithromycin [32].

Kanamoto et al. found that G. elegans gave a high level of susceptibility towards ampicillin (96.7%), amoxicillin (100%), imipenem (100%), and vancomycin (96.7%). In addition, G. elegans were susceptible to benzylpenicillin (82.7%), cefazolin (88.2%), ceftriaxone (76.4%), and minocycline (94.1%), with high resistance (93.3%) towards both of gentamicin and trimethoprim/sulfamethoxazole [33]. The results were similar to a study done by Dhotre et al., which revealed a resistance against penicillin, cefotaxime and cefitriaxone[32].

The results were close to a study by Basaranoglu et al., who found that S. mitis isolates showed moderate resistance to ampicillin, penicillin, cefotaxime and ceftriaxone [34]. Kim and Lee showed that S. mitis isolates were susceptible to amoxicillin, cefotaxime and vancomycin, with excellent resistance to ampicillin,

erythromycin and tetracycline [35]. Also, these results were similar to a study by Philips et al., which recorded high resistance rates towards ampicillin and was susceptible to both imipenem and erythromycin [36].

The current study's results were similar to those obtained by Tewari et al., who found substantial sensitivity rates to a range of antibiotics, including macrolides penicillins, fluoroquinolones, vancomycin and cephalosporins. At the same time, these bacteria presented high resistance rates to trimethoprim-sulfamethoxazole penicillin, ceftazidime, erythromycin, gentamicin, ceftriaxone, ciprofloxacin, amikacin, meropenem and imipenem, oxacillin [37]. Also, Szczerba found that K. kristinae isolates were highly resistant to ampicillin and erythromycin [38].

Conclusion

An interesting association between dental caries and tonsillitis in children aged 6-12 appeared. Moreover, it may be accepted that the infection passes much more frequently from the teeth to the tonsils than in the opposite direction; most Gram-positive and Gram-negative bacterial isolates were multi-drug resistant to antimicrobial agents.

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