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Full Length Research Paper

# Characteristics of *Streptococcus* and *Staphylococcus* strains isolated from acute cellulitis of dental origin in Ouagadougou, Burkina Faso

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Patients afflicted by acute cellulitis of dental origin are usually in need of urgent treatment. The most frequently isolated bacterial strains associated with this condition are Streptococcal and Staphylococcal species, which are also most commonly implicated with cellulitis in general. The aim of this study was to determine the antibiotic resistance profiles of Streptococcus and Staphylococcus isolated from patients with acute cellulitis of dental origin in a developing country such as Burkina Faso. Samples (exudates) taken from 52 patients (25 male [48.1%], 27 female [51.9%]) suffering from acute cellulitis were analyzed using conventional microbiology methods. Patients who were 19-40 years of age were the most commonly afflicted by acute cellulitis (representing 59.6% of the subjects in this study). Of the 52 samples taken, 25 (48.1%) were positive and 27 (51.9%) negative for Staphylococcus and/or Streptococcus. Seventeen Staphylococcus (32.7% of the samples) and 8 Streptococcus (15.4% of the samples) strains were isolated and characterized using antibiotic susceptibility profiling methods. All Streptococcus strains were found to be resistant to trimethoprim/sulfamethoxazole, the chloramphenicol, oxacillin, cefixim, cefuroxim, cefotaxim and ceftriaxon. The Staphylococcus strains were mostly resistant to cefixim (88.2%), piperacillin (70.6%), penicillin G (94.1%) and amoxicillin (76.5%). All strains were resistant to metronidazole. Given the high resistance of isolates to antibiotics, it may be necessary to assay bacterial antibiotic susceptibility patterns prior to prescribing these medications.

**Key words:** Acute cellulitis, tooth, *Streptococcus*, *Staphylococcus*, antibiotics, resistance, Ouagadougou, Burkina Faso.

# INTRODUCTION

Cervicofacial cellulitis is an inflammation of the fat cell tissues that entails an interesting head and neck anatomy which is often associated with microbial infections (Lakouichmi et al., 2014). Emergency diagnosis and therapy are generally necessary because the pathology's manifestation is usually not limited to a single area, and it tends to spread through tissue spaces to vital organs (Odzili et al., 2014). Furthermore, cervicofacial cellulitis is frequently associated with high mortality rates in sub-Saharan Africa (Odzili et al., 2014). Yet, despite its considerable morbidity and mortality, there have been few investigations of the etiology of this disease in Africa.

The most common form of cellulitis is a mixed infection (aerobic, facultative anaerobic and obligate anaerobes) which is of dental origin. Most treatments aim to eradicate the etiological agents of the disease. In most of these infections, the bacteria are part of the oropharyngeal flora, with the predominant genera being Gram-positive cocci such as *Streptococcus*, *Staphylococcus* and *Peptostreptococcus*, as well as Gram-negative bacilli (Oberoi et al., 2015).

Staphylococcus and Streptococcus are involved in several human infectious diseases, and they play an important role in the severity of the infections that they cause (Petti et al., 2014). The existence of multi-drug resistant (MDR) strains and the appearance of new resistance represent major challenges in the treatment of microbial infections and they have major implications regarding the choice of treatment (Kityamuwesi et al., 2015). Guidance for therapeutic decisions regarding the choice of antibiotic depends on the frequency of the bacteria isolated, and their sensitivity to different classes of antibiotics (Boisramé-Gastrin et al., 2011). There is ample evidence that antibiotic misuse is the most important risk factor for the development of bacterial resistance. Furthermore, an increase in the relative frequency of bacteria producing extended spectrum βlactamases (ESBL) has been reported both in hospitals and in the wider community. While exhibiting large geographical disparities, the spread of resistance is worldwide public health currently problem а (Laxminarayan and Heymann, 2012).

The acquisition of data on bacterial resistance to antibiotics is necessary in order to achieve better therapeutic management of infections, and to develop an antimicrobial resistance control strategies (Oberoi et al., 2015). This study aimed to determine the prevalence and antibiotic susceptibility of *Streptococcus* and *Staphylococcus* involved in acute cellulitis of dental origin in Burkina Faso.

### MATERIALS AND METHODS

### Study design and location

This was a prospective study conducted in Ouagadougou (Burkina Faso) (Figure 1) between June and October of 2014. Exudate samples were collected at the Municipal Center for Bucco-dental Health from patients suffering from acute cellulitis, and these were analyzed at the Laboratory of Molecular Biology, Epidemiology and Surveillance of Food-borne Bacteria and Viruses ("LaBESTA") at the University of Ouaga I Professeur Joseph KI-ZERBO School of Doctoral Science and Technology ("EDST") Centre for Research in Biological Sciences, Food and Nutrition ("CRSBAN").

### **Clinical data**

All patients gave informed consent to provide samples, for the epidemiological investigations, and to participate in the study. Data were collected using a standard form containing information regarding the patients' identity, medical history and dietary habits. Oral hygiene was assessed using the Björby and Löe's (1967) retention index, with a scale of 0-3 (Table 1). Upon clinical examination, written and image-based records of teeth affected by bacterial infection were compiled (for example, using panoramic or periapical radiography). Personal income levels were assessed by grouping patients into three occupational categories: low-income participants (for example, farmers, students, pupils and homemakers), high-income patients (for example, commercial and private sector employees) and moderate incomes (for example, public sector employees, informal sector workers, retirees and others similarly not in the work force). The type of food consumed was noted across four of the main food groups: meat products, seafood products, dairy products, sugar-based products and fruits and vegetables.

### Samples and processing

Fifty-two exudate samples were collected from patients presenting with acute cellulitis on an everyday basis over the study period (for 5 months). Patients with prior incidences of immunosuppressive diseases (for example, patients with HIV, cancer, diabetes, patients receiving corticosteroid therapy, etc.) were not excluded. Only participants with non-fistulized skin or oral mucosa cellulitis were included in the study (Figure 2). All other cases were excluded. Sampling was performed according to the method described by Rôcas and Sigueira (2013). Patients were asked to rinse their mouth for one minute with chlorhexidine (using a 0.12% solution). The inflated mucosa was then sanitized with 2% chlorhexidine solution prior to collection of up to 2 mL of exudate by piercing the infected area with a sterile needle (Figure 3). The exudates were then immediately transferred into a sterile tube containing thioglycollate resazurin broth (Liofilchem, Italy) (Figure 4). Tubes were conditioned in a cooler at 4°C and transported to the laboratory for microbiological analysis within two hours.

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Figure 1. Map of Kadiogo province with the study sites.

0	1			2	3	
Absence of tartar, tooth decay or fillings	Tooth fillings gum	decay close to	or the	Tooth decay, tartar, or filling in contact with the marginal gingiva, a degree of subgingival calculus	Tooth decay, tartar, or filling in the marginal gingiva, abundant subgingival calculus	

0 = Score of zero, 1 = score of one, 2 = score of two, 3 = score of three.



Figure 2. Cellulitis of dental origin.



Figure 3. Sampling of exudate.



Figure 4. Specimen storage.

# Isolation and identification of Streptococcus

Ten microlitres aliquots of anaerobically transported broth (thioglycollate resazurin) (Liofilchem, Italy) were streaked onto plates containing Columbia agar (Liofilchem, Italy) supplemented with hemoglobin (Liofilchem, Italy) and anaerobically incubated at 37°C for 48-72 h (Ellner et al., 1966). Colonies suspected to be *Streptococcus* (with small, white to grayish appearance) were then subcultured on Mueller-Hinton agar (Liofilchem, Italy) prior to biochemical confirmation of their identity using the API 20 Strep kit (bioMérieux, France). Interpretation of the results was done using

APIWEB V7.0 software (bioMérieux, France).

# Isolation and identification of Staphylococcus

Ten microlitres aliquots of anaerobically transported broth (thioglycollate resazurin) (Liofilchem, Italy) were streaked onto plates containing mannitol salt agar (Liofilchem, Italy) and anaerobically incubated at 37°C for 48-72 h (Chapman, 1945). Colonies suspected to be *Staphylococcus* (with a lush, pigmented appearance and surrounded by a yellow halo) were then subcultured on Mueller-Hinton agar (Liofilchem, Italy) and

characterized using the API Staph kit (bioMérieux, France). Interpretation of the results was done using APIWEB V4.1 software (bioMérieux, France).

### Antibiotic susceptibility testing

Antimicrobial susceptibility test was carried out using the agar disc diffusion method (Bauer et al., 1966); for Staphylococcus strains, Müller-Hinton agar (Liofilchem, Italy) was used; while for Streptococcus, Müller-Hinton agar (Liofilchem, Italy) supplemented with 5% defibrinated horse blood was used. The Müller-Hinton agar (Liofilchem, Italy) was inoculated with a 0.5 McFarland standard inoculum in each case. After depositing the antibiotics, plates were incubated anaerobically at 37°C for 24 h. The following 21 antibiotics were used: oxacillin (5 µg), amoxicillin (25 µg), amoxicillin-clavulanic acid (20+10 µg), cefotaxim (30 µg), cefuroxim (30 µg), cefixim (5 µg), ceftriaxon (30 µg), erythromycin (15 µg), trimethoprim/sulfametoxazole (1.25/23.75 µg), chloramphenicol (30 µg), gentamicin (30 µg), tobramycin (10 µg), netilmicin (30 µg), piperacillin (100 µg), piperacillin/tazobactam (100+10 µg), metronidazole (5 µg), penicillin G (10 IU), lincomycin (15 µg), spiramycin (100 µg), clindamycin (10 µg) and ciprofloxacin (5 µg) (Liofilchem, Italy). Inhibition zones were measured and bacterial strains classified as either 'resistant', 'intermediate sensitive', or 'sensitive' according to the European Committee of Antimicrobial Susceptibility Testing (EUCAST, 2014) guidelines, and those of the French Microbiology Society's Antibiogram Committee (CASFM, 2012).

### Statistical analysis

Statistical data analysis was performed using Epi-Info Version 7. The Chi-square test was used to determine the difference between two statistical variables. Differences were considered significant at p<0.05.

# RESULTS

### **Patient characteristics**

Patients in the study population were mostly (59.6%) in 19-40 years age bracket, with 51.9% being female and 48.1% male (Table 2). The first mandibular molar (50% of cases), second mandibular molar (9.7% of cases), and the first maxillary molar (7.8% of cases) were the most commonly affected by bacterial infection. Anamnesis revealed prior surgery (in 5.8% of cases) and hypertension (in 1.9% of cases); no other medical history was observed. Fish and meat items were the food products that were consumed the most, at 46.2 and 30.8% of total, respectively (p=0.0001) (Table 3). Lowincome participants represented the group most afflicted by this type of bacterial disease (57.7% of the study population; p=0.0009). The proportion of high-income patients with an oral infection was 19.2%, and those with moderate incomes: 23.1% of the study population. In terms of oral hygiene, 50 patients (96.2%; p=0.0001) were given a retention index score of 3; only 2 patients

Age group	Sex I			
(year)	Male	Female	10tal N (%)	
0-6	1 (4)	0 (0)	1 (1.9)	
7-12	3 (12)	2 (7.4)	5 (9.6)	
13-18	2 (8)	6 (22.2)	8 (15.4)	
19-40	14 (56)	17 (63)	31 (59.6)	
41-60	3 (12)	1 (3.7)	4 (7.7)	
> 60	2 (8)	1 (3.7)	3 (5.8)	
Total N (%)	25 (48.1)	27 (51.9)	52 (100)	

Table 2. Age and sex distribution of cellulitis cases.

(3.8 %) had a score of 1.

# **Bacterial etiologies**

Of the 52 samples collected, 25 (48,1%) tested positive and 27 (51.9%) tested negative for Staphylococcus and Streptococcus species (p>0.05). Seventeen were Staphylococcus (32.7% of the samples) and 8 were Streptococcus (15.4% of the samples) strains were isolated (p>0.05). Eight Staphylococcus species were Staphylococcus xylosus (n=6: isolated: 11.5%). Staphylococcus hominis (n=3; 5.8%), Staphylococcus lentus (n=2; 3.8%), Staphylococcus warneri (n=2; 3.8%), Staphylococcus saprophiticus (n=1: 1.9%). Staphylococcus cohnii ssp. cohnii (n=1; 1.9%). Staphylococcus haemolyticus (n=1; 1.9%) and Staphylococcus aureus (n=1; 1.9%). Five Streptococcus species and 2 subspecies of Streptococcus dysgalactiae were found: S. mitis (n=2; 3.8%), Streptococcus uberis 1.9%), Streptococcus dysgalactiae (n=1: SSD. dysgalactiae (n=1; 1.9%), Streptococcus pneumoniae (n=2; 3.8%), Streptococcus dysgalactiae ssp. equisimilis (n=1; 1.9%) and Streptococcus agalactiae (n=1; 1.9%). A single case (representing 4% of the study population) of dysgalactiae co-infection Streptococcus by ssp. dysgalactiae and Staphylococcus xylosus was identified.

### Antimicrobial susceptibility test

All *Streptococcus* strains were fully resistant to trimethoprim-sulfamethoxazole, chloramphenicol, oxacillin, cefixim, cefuroxim, cefotaxim and ceftriaxon. The *Streptococcus* strains also exhibited the following degrees of resistance to amoxicillin-clavulanic acid (83.3%), piperacillin-tazobactam (83.3%), ciprofloxacin (83.3%), penicillin G (83.3%) and amoxicillin (83.3%) (Figure 5A). The *Staphylococcus* species were largely resistant to cefixim (88.2%), piperacillin (70.6%), penicillin G (94.1%) and amoxicillin (76.5%) (Figure 5B). All the

Age group (year)	Meat products	Dairy products	Fish products	Sweet products	Fruits and vegetables
0-6	1 (1.9)	0 (0)	0 (0)	0 (0)	0 (0)
7-12	1 (1.9)	1 (1.9)	2 (3.9)	5 (9.6)	1 (1.9)
13-18	3 (5.8)	1 (1.9)	4 (7.7)	1 (1.9)	0 (0)
19-40	10 (19.2)	2 (3.9)	14 (26.9)	5 (9.6)	0 (0)
41-60	0 (0)	1 (1.9)	3 (5.8)	0 (0)	2 (3.9)
> 60	1 (1.9)	0 (0)	1 (1.9)	0 (0)	1 (1.9)
Total N (%)	16 (30.8)	5 (9.6)	24 (46.2)	7 (13.5)	4 (7.7)

**Table 3.** Dietary habits of the patients.



Figure 5. A: Antibiotic susceptibility of *Streptococcus* and B: susceptibility of *Staphylococcus*.

isolates were resistant to metronidazole (100%) (Table 4).

# DISCUSSION

This study showed that cervicofacial cellulitis of dental origin afflicts individuals of all ages; and those in the 19-40 years-old age group in particular, as they represented 59.6% of the total patient sample (Table 2). Similar prevalence was reported by others for this same age group (46.8% of the total) (Njifou et al., 2014).

In this study, 27 women (51.9%) and 25 men (48.1%) were afflicted by cellulitis of dental origin (p>0.05); which is similar to the result obtained by Miloundja et al. (2011), who found that 30 women (56%) and 25 men (43%) in their patient sample were afflicted. In a similar study, carried out in Morocco, a higher prevalence of dental cellulitis was reported in men (57%) when compared with the women (43%) (Rouadi et al., 2013).

The most frequently represented age group in this study was the one that also consumed the largest percentage of meat and fish products. An earlier study conducted in Ouagadougou, Burkina Faso by Barro et al. (2005) reported that these foods products were more likely to be contaminated with *Staphylococcus* and *Streptococcus*. This could hence well explain the bacterial etiology of cellulitis of dental origin that can be greatly exacerbated by poor oral hygiene and by pre-existing carious lesions that can serve as receptacles. The 19-40 years group was comprised mainly of students and low to mid-level employees. They may pay less attention to their diet, have an affinity for fast food, and tend not to heed oral hygiene recommendations. These factors may underlie the high percentage of cellulitis in this age group.

Several authors have established that *Staphylococcus* is carried as a commensal microorganism on the skin and nasal passages of humans and animals (Hanning et al., 2012). Humans can become contaminated by these pathogens through direct contact with animals, while animal feces can also contaminate dam water intended for human consumption (Mehanned et al., 2014). There are additional studies that suggest that the risk of environmental contamination and infection in dental healthcare settings may be quite considerable (Petti et al., 2014); *S. aureus*, and its carriers, are sources of healthcare-associated infections, and these can hence occur in dental healthcare settings. Dental therapy may promote the dissemination of airborne human bacteria in

	Susceptibility of bacteria isolated N (%)								
Antibiotics		Streptococcus		Staphylococcus					
	R		S	R	I	S			
AUG	7 (87.5)	0(0)	1 (12.5)	8 (47.1)	0 (0)	9 (52.9)			
CRO	8 (100)	0 (0)	0 (0)	8 (47.1)	4 (23.5)	5 (29.4)			
CFM	8 (100)	0 (0)	0 (0)	15 (88.2)	0 (0)	2 (11.8)			
CXM	8 (100)	0 (0)	0 (0)	5 (29.4)	2 (11.8)	10 (58.8)			
СТХ	8 (100)	0 (0)	0 (0)	5 (29.4)	6 (35.3)	6 (35.3)			
CN	3 (37.5)	1 (12.5)	4 (50)	9 (52.9)	0 (0)	8 (47.1)			
CD	4 (50)	1 (12.5)	3 (37.5)	11 (64.7)	2 (11.8)	4 (23.5)			
LZ	8 (100)	0 (0)	0 (0)	17 (100)	0 (0)	0 (0)			
TZP	7 (87.5)	0 (0)	1 (12.5)	4 (23.5)	0 (0)	13 (76.5)			
OX	8 (100)	0 (0)	0 (0)	10 (58.8)	0 (0)	7 (41.2)			
SP	4 (50)	2 (25)	2 (25)	7 (41.2)	7 (41.2)	3 (17.6)			
MY	4 (50)	3 (37.5)	1 (12.5)	11 (64.7)	3 (17.6)	3 (17.6)			
PRL	6 (75)	0 (0)	2 (25)	12 (70.6)	0 (0)	5 (29.4)			
ТОВ	6 (75)	0 (0)	2 (25)	11 (64.7)	0 (0)	6 (35.3)			
NET	5 (62.5)	1 (12.5)	2 (25)	10 (58.8)	0 (0)	7 (41.2)			
E	5 (62.5)	1 (12.5)	2 (25)	10 (58.8)	3 (17.6)	4 (23.5)			
SXT	8 (100)	0 (0)	0 (0)	8 (47.1)	3 (17.6)	6 (35.3)			
С	8 (100)	0 (0)	0 (0)	11 (64.7)	0 (0)	6 (35.3)			
CIP	7 (87.5)	0 (0)	1 (12.5)	5 (29.4)	0 (0)	12 (70.6)			
Р	7 (87.5)	0 (0)	1 (12.5)	16 (94.1)	0 (0)	1 (5.9)			
AML	7 (87.5)	0 (0)	1 (12.5)	13 (76.5)	0 (0)	4 (23.5)			

Table 4. Antibiotic susceptibility of Streptococcus and Staphylococcus strains.

AUG = amoxicillin/clavulanic-acid, CRO = ceftriaxon, CFM = cefixim, CXM = cefuroxim, CTX = cefotaxim, CN = gentamycin, CD = clindamycin, LZ = metronidazole, TZP = piperacillin/tazobactam, OX = oxacillin, SP = spiramycin, MY = lincomycin, PRL = piperacillin, TOB = tobramycin, NET = netilmicin, E = erythromycin, SXT = trimethoprim/sulfamethoxazole, C = chloramphenicol, CIP = ciprofloxacin, P = penicillin G, AML = amoxicillin, R = resistant, I = intermediate, S = sensitive. Strains categorized as "S" are those for which the probability of therapeutic success is strong in the case of a systemic treatment with the recommended dosage provided in the summary of the product characteristics (SmPC), written by the French Agency Health Safety of Health Products (AFSSAPS). Strains categorized as "R" are those with a high probability of treatment failure regardless of the type of treatment and antibiotic's dose used. Strains categorized "I" are those with therapeutic success is unpredictable.

the environment (Messano et al., 2013). Indeed, Staphylococci (S. aureus, and even methicillin-resistant S. aureus, MRSA) have been detected with high-speed instruments during dental therapy (Kimmerle et al., 2012). Since S. aureus, and also occasionally MRSA, can be detected in the dental environment (Petti and Polimeni, 2012), contamination seems to be caused mostly by contact with hands. Our socio-economic data showed that low-income patients were the most frequently represented group in this study (57.7%). This result may be explained by the fact that most of these participants (n=50; 96.2%) have poor oral hygiene (p=0.0001), and that they lack the required financial resources to obtain timely treatment. This poor level of hygiene also appears to be linked to a general disregard for oral hygiene practices. The present study showed, however, that despite having poor oral hygiene, the 19-40 age group engaged in a consistent brushing regimen; with daily brushing of 2 to 3 times a day. Lack of effectiveness and irregularly brushing of the mouth could however explain this prevalence.

Microbiological analysis showed that 17 strains of Staphylococcus (32.7% of the samples) and 8 strains of Streptococcus (15.4% of the samples) were isolated from the total of 52 samples that were collected. Others have also reported the involvement of Staphylococcus and Streptococcus in cellulitis of dental origin (Miloundja et al., 2011), including some studies in Cameroon (Niifou et al., 2014; Kityamuwesi et al., 2015). Eight Staphylococcus species, especially Staphylococcus warneri were isolated in this study. As a common saprophyte of human epithelia, Staphylococcus warneri is frequently isolated from saliva, dental plaques and nasal swabs. Indeed, it represents the third most prevalent coagulase-negative Staphylococcus species after S. epidermidis and S. hominis (Ohara et al., 2008). In light of the progressive refinement of identification techniques over the last three decades, S. warneri has increasingly emerged as a new pathogenic species that is capable of causing serious infections, usually in association with the presence of

implant materials (Campoccia et al., 2010). The mouth, by virtue of its constant temperature, and the presence of many food fragments and metabolites, is an ideal culture medium for these bacterial species. Thus, poor oral hygiene readily permits the multiplication of oral microbiota (Lam et al., 2012).

All Streptococcus strains were fully resistant to trimethoprim-sulfamethoxazole, chloramphenicol, oxacillin, cefixim, cefuroxim, cefotaxim and ceftriaxon. The Staphylococcus species were largely resistant to cefixim (88.2%), piperacillin (70.6%), penicillin G (94.1%) and amoxicillin (76.5 %). All isolates (Streptococcus, Staphylococcus) were resistant to metronidazole (100%). Similar to what has been reported in previous studies 2015), (Oberoi al., the Streptococcus et and Staphylococcus isolates in this study were highly resistant to β-lactam antibiotics. β-Lactam antibiotics are a major class of antibiotics that are used widely in clinical practice. Development of antibiotic resistance in bacteria is a natural phenomenon, but high-level resistance is exacerbated by the overuse of antibiotics (Oberoi et al., 2015). Furthermore, resistant strains appear to be the dominant forms, and this is the result of selection pressure following exposure to the antibiotic (Oberoi et al., 2015). Staphylococcus and Streptococcus strains are opportunistic pathogens, commensal on the human body. Yet, this study revealed that these strains are not only involved in cellulitis of dental origin in Burkina Faso, also they exhibit multi-resistance to common antibiotics. This indicates that this burgeoning problem needs to be given due consideration by healthcare policymakers.

Metronidazole is an anti-parasitic and antibiotic agent that is used to treat infections caused by parasites and obligate anaerobic bacteria (Audu et al., 2012). The resistance to metronidazole reported in the strains isolated in this study may be due to the fact that these strains are not obligate anaerobes. 'Natural resistance' is a chromosomal property, present in all strains of the same species or the same bacterial genus, which influences sensitivity towards an antibiotic. Streptococcus is naturally resistant to sodium azide, crystal violet, nalidixic acid, polymixims and aminoglycosides (low level natural resistance). Natural resistance in Staphylococcus is rare, although natural resistance to guinolones does occur. The isolates from this study (Staphylococcus and Streptococcus) were resistant to metronidazole, which is an antibiotic and antiparasitic agent that belongs to the nitroimidazoles group. It could be possible that the strains exhibited natural resistance to low-doses (for example, 5 µq) of metronidazole.

### Conclusion

The present study further showed that all cases of cervicofacial tumefactions should receive thorough

medical attention. Cellulitis of dental origin is caused by the proliferation of aerobic and anaerobic bacteria present in the oral flora, and it is generally initiated by decay or pulpar necrosis. In this study, it was observed that *Staphylococcus* strains were the most frequently involved in acute cellulitis, albeit with acceptable levels of antibiotic susceptibility. On the other hand, although *Streptococcus* strains were less often involved, they were resistant to a greater diversity of antibiotics. Fortunately, accurate diagnosis combined with efficient antibiotherapy and surgical treatment (avulsion of the causal tooth and purulent collection's drainage) enables healthcare professionals to achieve a cure in most cases.

# Conflict of Interests

The authors have not declared any conflict of interest.

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