Occurrence and Antibiotic Susceptibility of *Aerococcus* and *Enterococcus* Strains Isolated from Acute and Chronic Cellulites of Dental Origin in Ouagadougou, Burkina Faso

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Abstract

Cellulites of dental origin are extensive polymicrobial infections of the face and neck cell-adipose tissues. Management of infections of this nature presents significant challenges. The key to this is the identification of the causative organisms and determination of their susceptibility to antibiotics. *Enterococcus* and *Aerococcus* are pathogenic and opportunistic bacteria. Despite their low virulence, these microorganisms are responsible for many human infections. A major problem with these bacteria is their frequent multidrug resistance. The lack of data on these bacterial strains involved in the cellulite in Burkina Faso thus justifies the choice of *Enterococcus* and *Aerococcus*. This study was therefore undertaken to determine bacterial etiologies and antibiotic resistance profiles associated with cellulites cases in Ouagadougou, Burkina Faso. A total of 62 samples from patients were analyzed. The isolates were obtained using conventional microbiology procedures. Strains were identified by using API 20 Strep (bioMérieux, France). Antibiotic susceptibility and detection of extended spectrum β-lactamases (ESBLs) were performed according to the European Committee of Antimicrobial Susceptibility Testing (EUCAST). The patients were 41.9% males and 58.1% females. *Aerococcus* was isolated from 30 patients and *Enterococcus* from 7. *Aerococcus* strains were resistant to metronidazole (100%), cefixim (93.3%), trimethoprim-sulfamethoxazole (86.7 %), oxacillin (83.3%), penicillin G (83.3%), cefotaxime (80%), chloramphenicol (80%), cefuroxime (76.7%) and to ceftriaxone (76.7%). *Enterococcus* strains were resistant to metronidazole (100%), cefixim (100%), cefotaxim (100%), ceftriaxone (100%), cefuroxime (100%), trimethoprim-sulfamethoxazole (100%), penicillin G (100%), oxacillin (100%) and to amoxicillin (100%). Only one *Aerococcus viridans* strain was positive for ESBL production (2.7%). The emergence of resistance among the bacterial strains that have been implicated in odontogenic infections is a public health issue in Burkina Faso that warrants a significant degree of concern.

Keywords: Cellulitis; Tooth; *Aerococcus*; *Enterococcus*; Antibiotic; Resistance; Ouagadougou; Burkina Faso

Introduction

Pathologies with a dental origin often result in infectious complications, which can be local, regional, or general. The high incidence of infectious complications represents a significant public health issue that is all the more unacceptable given that effective prevention is generally within reach [1]. These types of infections are frequently associated with poor oral hygiene [2-4]. Tooth decay is an affliction that currently occurs worldwide [5]. The decay arises due to microbial proteolysis of enamel and dentin related to the development and stagnation of the dental plaque. The oral cavity provides ideal conditions for microbial growth (i.e. the right humidity, temperature, etc.), and the flora is a combination of aerobic and anaerobic bacteria. These are often non-pathogenic commensal organisms, although some, such as *Streptococcus*, *Peptostreptococcus*, *Bacteroides*, etc. are opportunistic and can become pathogenic [6,7]. Facial cellulites largely constitute the main complication of dental diseases, and they are the leading cause for emergency dentistry and maxillofacial surgery [6]. Cellular tissue is partitioned into regions of muscles and musculo-aponeurotic bulkheads that fit on the maxilla and mandible [4]. These areas or facial cell-fat sections are in continuity with the submandibular region and the rest of the cervical region. Cervical areas communicate with mediastinal regions. Germs that reach the apex and the periapical dental organ through the bone and the periosteum gain access to the oro-facial areolar tissue, as the cellulitis progresses beyond the acute stage. Chronicity can set in if the treatment is not sufficiently rigorous. Severe neck cellulitis then becomes a distinct possibility, and this can be life-threatening [3,8]. Management of this condition is challenging, as it is both expensive and difficult. Identification of the causative organisms is the key to treatment [8]. The percentage of deaths as a result of this pathology has been reported to range from between 7% and 50% [4,9]. The infection is however a mixed polymicrobial [10,11], and there is a general consensus regarding the predominance of anaerobes [12]. *Aerococci* and *Enterococci* are normal human commensals and ecologically complex environment of the oral cavity [13-15]. They are pathogenic and opportunistic bacteria [14,16,17]. *Aerococcus* and *Enterococcus* also occasionally cause systemic infections in immunocompromised hosts, including meningitis, urinary tract infections, osteomyelitis, arthritis, wound infections, and, most commonly, bacteremia and endocarditis [14,18]. Oral *Aerococcus* and *Enterococcus* may be a potential reservoir for the transferable elements of virulence and antimicrobial resistance [14,15]; severe cellulitis typically involves microbial virulence with the presence of multi-resistant germs. The treatment is medical and surgical, and sometimes associated with an appropriate form of resuscitation [19]. Antibiotic therapy should be thorough and focused. In the first instance it is probabilistic in nature and aimed at targeting *Streptococcus* and anaerobes. It is then adapted to the antibiogram data [20,21]. The outcome of severe cellulitis prognosis is related mainly to the affected site, to the early and effective initial treatment. Isolation of the causative organism is a critical step in management of the condition [4,9,22]. The present study aimed to determine the prevalence and antimicrobial susceptibility of *Enterococcus* and *Aerococcus* strains involved in these infections, in order to better prevent them.

Materials and Methods

Study design and site

This was a prospective study conducted in the Centre Municipal de Santé Bucco-Dentaire de Ouagadougou, Burkina Faso (Figure 1) from June of 2014 to October of 2014.
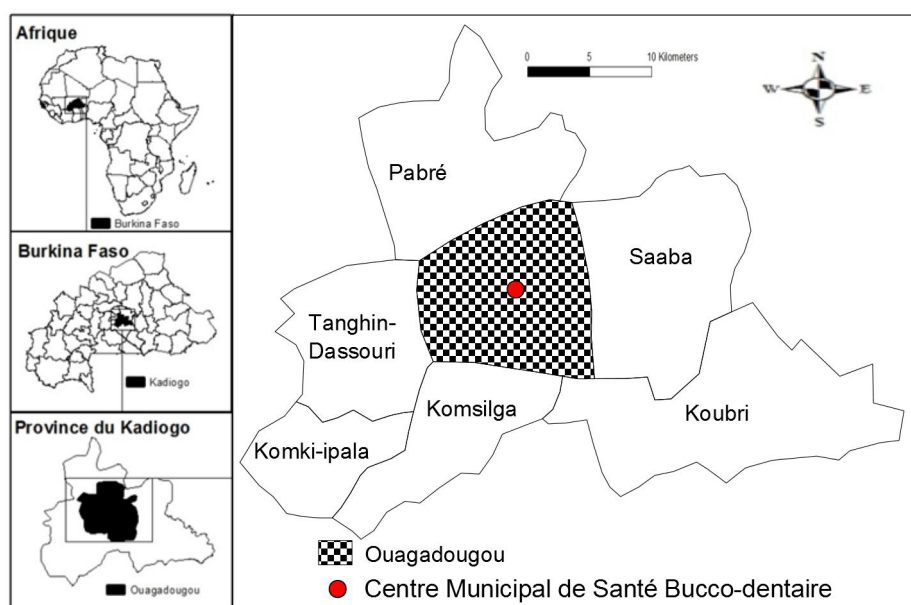

Figure 1: Map of Kadiogo Province with the study sites indicated

Clinical data

All samples and epidemiological investigations were carried out with the informed consent of the patients. An investitive data collection sheet was prepared. The main data collected included personal information (e.g. age, sex, occupation, etc.), clinical information (i.e. medical history, oral examinations), and dietary habits. Oral hygiene was assessed using the retention index of Björby and Löe, [23], as a score from 0-3 (Table 1).

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

Legend: 0 = Score of zero, 1 = Score of one, 2 = Score of two, 3 = Score of three
Table 1: Oral hygiene index

Samples and their processing

Sixty two (62) samples were collected. The study population was comprised of patients of both sexes and of all ages who were seeking treatment for acute or chronic cellulitis on permanent or temporary teeth. Only non-fistulized cellulites of the skin or oral mucosa were taken into account. All other cases were excluded. After the clinical diagnosis of cellulitis of dental origin, sampling was performed according to the Rôcas and Siqueira, [24] method. Thus, each patient rinsed his mouth for a few seconds with 0.12% chlorhexidine prior to sampling. The inflated mucosa was sanitized with 2% chlorhexidine solution. Two (2) mL of exudate was then aspirated using a sterile mounted syringe. This exudate was immediately transferred to a sterile tube containing the anaerobic transport and enriched broth (Thioglycollate resazurin) (Liofilchem, Italy). Tubes were chilled to 4 °C in a cooler and then transported immediately to the laboratory for microbiological analyzes within two hours.

Isolation and identification of *Aerococcus* and *Enterococcus*

In the laboratory, steriles tubes containing samples were incubated at 37 °C for 2 hours for enrichment. After 2 h incubation in thioglyconate resazurin broth (bioMérieux, France), ten (10) µl aliquot of enriched broth was streaked onto Columbia agar (Liofilchem, Italy) supplemented with hemoglobin (Liofilchem, Italy) and onto Bile Esculin Azide (BEA) agar (bioMérieux, France) plates and incubated anaerobically at 37 °C for 48 to 72 hours. Two or three greyish-white to greenish small colonies on the Columbia agar (Liofilchem, Italy) and presumed to be *Aerococcus* were subcultured on Mueller-Hinton agar (Liofilchem, Italy). Two to three colonies on the BEA agar that were suspected of being *Enterococcus* (small translucent colonies with a black halo) were also collected and subcultured on Mueller-Hinton medium (Liofilchem, Italy). After 24 hours of incubation at 37 °C, suspected colonies of *Aerococcus* and *Enterococcus* were tested for their positive Gram stain and catalase reaction (bioMérieux, France). Species biochemical identification was performed with the API 20 Strep (bioMérieux, France) according to the manufacturer's recommendation, and the interpretation was carried out using APIWEB V7.0 software (bioMérieux, France).

Antimicrobial susceptibility testing

Antimicrobial resistance of the isolated *Aerococcus* and *Enterococcus* strains was determined by using the agar disc diffusion method according to the recommendations of the European Committee of Antimicrobial Susceptibility Testing (EUCAST) [25]. Twenty one (21) different antibiotics were used: oxacillin (5 µg), amoxicillin (30 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 (10 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), ciprofloxacin (5 µg). Diameters of the inhibition zones were determined according to EUCAST, [25], and strains were classified as being either "resistant", "intermediate sensitive" or "sensitive".

Detection of Extended Spectrum β-lactamases (ESBLs) producing strains

Strains resistant to β-lactam antibiotics were tested to examine ESBL production, as recommended by EUCAST, [25]. This test is based on the detection of synergy between an amoxicillin/clavulanic-acid disc and two discs of third generation cephalosporins (ceftriaxone and cefotaxime) separated by 2 to 3 cm from one another. The presence of ESBL is revealed by the appearance of synergy between the discs, giving the appearance of "champagne cork" shape.

Statistical analysis of the data

The statistical analyses of the data were performed using Sphinx version 5 software. The Chi-square test (Chi^2) was used for the comparison of two variables. Differences were considered significant at $p < 0.05$.

Results

Characteristics of patients

The data collected showed that the 19-40 age group is highly represented in this patient population (i.e. 59.7% of the total) (Table 2). This study showed that 56.5% of the patients were low-income earners (e.g. farmers, students, pupils or homemakers). High-income patients (e.g. commercial and private employees) and those with modest incomes (e.g. public employees, informal sector workers, retirees and others) accounted for 21% and 22.6%, respectively. Sixty patients (96.8%) were ranked in level 3, and only 2 patients (3.2%) in level 1, of the Björby and Löe, [23], retention index. Most patients practiced self-medication (72.6%) (p = 0.0004). The most common antibiotics consumed were: amoxicillin, amoxicillin-clavulanic acid, rovamycin, flagyl, ampicillin, erythromycin, trimethopim-sulfametoxazole, metronidazole and ciprofloxacin. Nothing was revealed about the medical history of patients. No patient reported the presence of diabetes or an immunosuppressive disease. Patients were unaware of their general condition (90.4%). Only 1 reported hypertension and 4 surgical history.

Age group (years)	Sex n (%)		Total N (%)
	Male	Female	
0-6	1 (1.6)	0 (0)	1 (1.6)
7-12	3 (4.8)	2 (3.2)	5 (8.1)
13-18	2 (3.2)	7 (11.3)	9 (14.5)
19-40	15 (24.2)	22 (35.5)	37 (59.7)
41-60	3 (4.8)	2 (3.2)	5 (8.1)
>60	2 (3.2)	3 (4.8)	5 (8.1)
Total N (%)	26 (41.9)	36 (58.1)	62 (100)

Table 2: Age and sex distribution of cellulitis cases

Bacterial etiologies

Sixty two (62) samples were collected, including 52 acute cellulites (83.9%) and 10 chronic cellulites (16.1%). Thirty seven (37) pathogens were isolated, including 30 strains of *Aerococcus* (81.1%) and 7 strains of *Enterococcus* (18.9%) (Table 3).

Pathologies	Isolated bacteria n (%)				
	Aerococcus		*Enterococcus*		
	viridans	*urinae*	*faecium*	*avium*	*Faecalis*
Acute cellulitis	22 (59.5)	3 (8.1)	2 (5.4)	3 (8.1)	0 (0)
Chronic cellulitis	4 (10.8)	1 (2.7)	1 (2.7)	0 (0)	1 (2.7)
Total N (%)	26 (70.3)	4 (10.8)	3 (8.1)	3 (8.1)	1 (2.7)

Table 3: Isolated pathogens

Antimicrobial susceptibility testing

Aerococcus strains exhibited resistance to metronidazole (100%), cefixim (93.3%) trimethoprim-sulfamethoxazole (86.7%), oxacillin (83.3%), penicillin G (83.3%), cefotaxim (80%), chloramphenicol (80%), cefuroxim (76.7%) and to ceftriaxone (76.7%) (Table 4). *Enterococcus* strains were highly resistant to metronidazole (100%), cefixim (100%), cefotaxim (100%), ceftriaxone (100%), cefuroxim (100%), trimethoprim-sulfamethoxazole (100%), penicillin G (100%), oxacillin (100%) and to amoxicillin (100%) (Table 5). A single strain of *Aerococcus viridans* was positive for ESBL production (2.7%).

Antibiotics	Susceptibility of the isolated bacteria N (%)		
	Resistant	Intermediate	Sensitive
Amoxicillin-Clavulanic acid	19 (63.3)	0 (0)	11 (36.7)
Ceftriaxon	23 (76.7)	2 (6.7)	5 (16.7)
Cefixim	28 (93.3)	0 (0)	2 (6.7)
Cefuroxim	23 (76.7)	0 (0)	7 (23.3)
Cefotaxim	24 (80)	1 (3.3)	5 (16.7)
Gentamycin	7 (23.3)	1 (3.3)	22 (73.3)
Clindamycin	17 (56.7)	3 (10)	10 (33.3)
Metronidazole	30 (100)	0 (0)	0 (0)
Tazobactam-piperacillin	15 (50)	0 (0)	15 (50)

Antibiotics	Susceptibility of the isolated bacteria N (%)		
	Resistant	Intermediate	Sensitive
Oxacillin	25 (83.3)	0 (0)	5 (16.7)
Spiramycin	19 (63.3)	5 (16.7)	6 (20)
Lincomycin	21 (70)	5 (16.7)	4 (13.3)
Piperacillin	20 (66.7)	0 (0)	10 (33.3)
Tobramycin	19 (63.3)	4 (13.3)	7 (23.3)
Netilmicin	14 (46.7)	8 (26.7)	8 (26.7)
Erythromycin	20 (66.7)	7 (23.3)	3 (10)
Trimethoprim-Sulfamethoxazole	26 (86.7)	1 (3.3)	3 (10)
Chloramphenicol	24 (80)	0 (0)	6 (20)
Ciprofloxacin	18 (60)	2 (6.7)	10 (33.3)
Penicillin	25 (83.3)	0 (0)	5 (16.7)
Amoxicillin	21 (70)	0 (0)	9 (30)

Table 4: Evaluation of the sensitivity of *Aerococcus* isolates to antibiotics

Antibiotics	Susceptibility of the isolated bacteria N (%)		
	Resistant	Intermediate	Sensitive
Amoxicillin-Clavulanic acid	5 (71.4)	0 (0)	2 (28.6)
Ceftriaxon	7 (100)	0 (0)	0 (0)
Cefixim	7 (100)	0 (0)	0 (0)
Cefuroxim	7 (100)	0 (0)	0 (0)
Cefotaxim	7 (100)	0 (0)	0 (0)
Gentamycin	2 (28.6)	0 (0)	5 (71.4)
Clindamycin	6 (85.7)	0 (0)	1 (14.3)
Metronidazole	7 (100)	0 (0)	0 (0)
Tazobactam-piperacillin	4 (57.1)	0 (0)	3 (42.9)
Oxacillin	7 (100)	0 (0)	0 (0)
Spiramycin	4 (57.1)	2 (28.6)	1 (14.3)
Lincomycin	6 (85.7)	1 (14.3)	0 (0)
Piperacillin	6 (85.7)	0 (0)	1 (14.3)
Tobramycin	6 (85.7)	0 (0)	1 (14.3)
Netilmicin	5 (71.4)	0 (0)	2 (28.6)
Erythromycin	4 (57.1)	3 (42.9)	0 (0)
Trimethoprim-Sulfamethoxazole	7 (100)	0 (0)	0 (0)
Chloramphenicol	5 (71.4)	1 (14.3)	1 (14.3)
Ciprofloxacin	2 (28.6)	0 (0)	5 (71.4)
Penicillin	7 (100)	0 (0)	0 (0)
Amoxicillin	7 (100)	0 (0)	0 (0)

Table 5: Evaluation of the sensitivity of *Enterococcus* strains to antibiotics

Discussion

This study showed that head and neck cellulitis of dental origin can afflict all age groups. The incidence of cellulitis (acute and chronic) of dental origin was shown to depend significantly on age (p=0.0001). Thus, we found that the most highly affected age group was comprised of individuals in the 19-40 years of age group, representing 59.6% of cases (Table 1). A prior study in French hospital (Dijon) found that the 19-36 years of age group was the most affected (as 51.7% of total) [2]. Lakouichmi, *et al*. [6], showed that the average age reached was 35 years. Njifou Njimah, *et al*. [1] also found that in Algeria the 21-30 years age group was the most affected (46% of total). The high prevalence of cellulitis of dental origin is linked to the socio-economic conditions of the patients [16]. Based on the results of this study, this dependence is significant (p = 0.0006). Notably, the survey showed that low-income patients were the largest group (at 56.5% of total). Most patients may not have the financial means to afford treatment for early-stage oral diseases. This pathology hence appears to be a disease that mainly afflicts the lower economic sector of our society.

Twenty six (26) men (41.9%) and 36 women (58.1%) had sought a medical consultation in light of cellulitis of dental origin. The results of the statistical analysis showed that the cellulitis of dental origin does not depend significantly on gender ($p > 0.05$). Similar results, i.e. 14 men (45%) and 18 women (56%), were found in Libreville, Gabon [4]. The same observation was made in Morocco, with 28 men (56.4%) and 22 women (43.6%) being afflicted [10]. At the ORL department and Maxillofacial Surgery Hospital of Rabat-Salé CHU, Morocco, El Ayoubi, *et al.* [11], found 53% of men and 47% of women were afflicted by this condition. By contrast, some authors found a predominance of the disease among men [4].

Based on the results of the survey, it would appear that the high incidence of odontogenic cellulitis is due to poor oral hygiene ($p = 0.0001$). The same observation was made by several other authors [2-4]. Poor hygiene has also been linked to a lack of education, and this study population is indeed made up mainly of farmers and housewives. The lack of effectiveness of brushing and/or its irregularity could also explain the high prevalence of poor oral hygiene (96.8% of patients) ($p = 0.0001$) among this patient cohort. Drainage and/or surgical debridement are central to the treatment of cellulitis of dental origin. A wide incision should enable flattening of all of the cellulitis areas [2]. Bacteriological identification and treatment based on a dental cause is however paramount [4]. The initial site of the infection is dental or peri-dental in 80% of cases [22].

The microbiological analysis results showed that 37 pathogens, including 30 strains of *Aerococcus* (81.1%) and 7 strains of *Enterococcus* (18.9%) were isolated and identified. There were also significant differences ($p = 0.0024$) between the isolated genus. Among the 30 strains of *Aerococcus*, *Aerococcus viridans* (83.3%) was mainly involved in the cellulitis of dental origin. The normal habitat of *Aerococcus viridans* is the natural environment [13]. However, in humans, it can be isolated from blood cultures (e.g. endocarditis, bacteremia), joint fluid, meningitis, and skin wounds [16]. This bacterium is often regarded as the cause of nosocomial infections, and it has been isolated from odontogenic infections. For a long time it has been confounded with *Streptococcus* or *Staphylococcus*. This germ has been described as such due to the very similar characteristics in terms of morphology and size, and it has hence been scantly reported in the literature [17]. Jiang, *et al.* [18], reported severe odontogenic infections due to *Aerococcus viridans*. A study in Tunisia noted involvement of *Enterococcus* in oral infections [14]. These bacteria are opportunistic pathogens, and they have been implicated in severe infections in immunocompromised as well as immunocompetent patients [13].

In our study, *Aerococcus* exhibited resistance to metronidazole (100%), cefixim (93.3%) trimethoprim-sulfamethoxazole (86.7%), oxacillin (83.3%), penicillin G (83.3%), cefotaxim (80%) chloramphenicol (80%), cefuroxim (76.7%) and ceftriaxone (76.7%). However, this germ exhibited good sensitivity to clyndamicin (73.3%). Despite the low prevalence of *Enterococcus* strains, these strains were highly resistant to metronidazole (100%), cefixim (100%), cefotaxim (100%), ceftriaxone (100%), cefuroxim (100%), trimethoprim-sulfamethoxazole (100%), penicillin G (100%), oxacillin (100%) and amoxicillin (100%). A study in Tunisia noted resistance of *Enterococcus* to penicillin (100%), trimethoprim-sulfamethoxazole (71%) and amoxicillin (29%) [14]. Several other studies have also reported high resistance of *Enterococcus* to antibiotics [15]. These pathogens hence represent considerable health concerns, especially in developing countries. Their resistance could be due to the bacterium having specific virulence factors, such lipoteichoic acid, pheromones, lytic enzymes and cytolysin [15]. We also found that all of the isolated *Enterococcus* strains were resistant to β-lactam antibiotics. Such resistance is generally linked to production of β-lactamases by the strain in question [21]. Our results could be explained by this phenomenon. However, out of the 37 strains, only one (i.e 2.6%) *Aerococcus viridans* produced ESBL. This low rate of ESBL supports the hypothesis that inadequate and often indiscriminate use of antibiotics may lead to other forms of resistance. Kayaoglu and Orstavik, [15], argue that *Enterococcus* is a very virulent bacterium that is difficult to eradicate through use of antibiotics.

Conclusion

Cellulitis of dental origin remains a common pathology that is a significant public health issue. It is a potentially severe disease for which treatment is expensive. In light of pronounced socioprofessional and economic impacts, there is a clear need for effective and targeted prevention policies aimed at reducing the morbidity and mortality associated to these infections. Severe cellulitis is characterized by microbial virulence with the presence of a multi-resistant germ antibiogram. It is important to always look for, identify, and assess their sensitivity to antibiotics.

Conflict of Interest

The authors have not declared any conflict of interests.

Acknowledgements

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