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Detection, characterization, and antimicrobial susceptibility of *Globicatella sanguinis* isolated from endodontic infections in Ouagadougou, Burkina Faso

Wendpoulomdé Aimé Désiré Kaboré,[1] Simavé René Dembélé,[1] Nicolas Barro[1]

[1]Laboratory of Molecular Biology, Epidemiology and Surveillance of Bacteria and Viruses Transmitted by Food/Center for Research in Biological, Food and Nutritional Sciences/Graduate School of Science and Technology/ Joseph KI-ZERBO University, Ouagadougou, Burkina Faso

Purpose: *Globicatella sanguinis* is an emerging pathogen rarely recognized as a cause of infection. The objective of this study was to investigate the role of this bacterium in endodontic infections in Burkina Faso and to determine its susceptibility to antibiotics.

Methods: This cross-sectional descriptive study was conducted at the Municipal Center of Oral Health of Ouagadougou from June to October 2014. Clinical data were collected using a sheet. Bacteria were isolated by streaking method on selective medium, and identification was done by API 20 Strep gallery. Antibiotic susceptibility was determined by the diffusion method on solid medium.

Results: A total of 125 patients were within aged 19–40 years (55.2%). Apical periodontitis accounted for 50.4%, and endodontic cellulitis accounted for 49.6% of endodontic infections. Five isolates of G. *sanguinis* have been identified. They were resistant (100%) to cefotaxime, metronidazole, and penicillin *G. Spiramycin* showed an intermediate sensitivity of 60%. Isolates showed good sensitivity (100%) to trimethoprim–sulfamethoxazole, amoxicillin–clavulanic acid, and tazobactam–piperacillin. One of them produced extended spectrum β-lactamases.

Conclusion: The severity of infections caused by *G. sanguinis* reflects difficulties to eradicate these bacteria from the root canal system.

Keywords: *Globicatella sanguinis*, endodontic infections, antibiotic resistance.

Introduction

Bacteria of *Globicatella genus* are Gram-positive cocci, facultative anaerobic, non-alpha hemolytic, and catalase negative. Morphologically, these bacteria are close to *Streptococcus* and *Aerococcus* that often create confusion in their identification by commercial phenotypic methods (1). *Globicatella sanguinis* is an emerging pathogen that is increasingly implicated in human infections particularly in the blood, urinary tract, and central nervous system (2,3). *G. sanguinis* was described in 1992 as a new species when Collins et al. characterized several isolates showing a phenotypic resemblance to *Streptococcus uberis* (4). The anatomical situation and the physiological role

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Correspondence: Wendpoulomdé Aimé Désiré Kaboré. Laboratory of Molecular Biology, Epidemiology and Surveillance of Bacteria and Viruses Transmitted by Food/Center for Research in Biological, Food and Nutritional Sciences/Graduate School of Science and Technology/Joseph KI-ZERBO University, Ouagadougou, Burkina Faso.
Tel: +22625333434 e-mail: dr_kabore@yahoo.fr
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of the oral cavity make it one of the most skeptical sites in the human body. The oral cavity contains a very variable dense microbial flora. Approximately 700 bacterial species have been identified in the oral microbiome (5). These very diverse flora may develop in different surfaces in the mouth. These bacteria are commensal and resident. They maintain a symbiotic relationship with the host and can be opportunistic. Endodontic infections (apical periodontitis and cellulitis) are generally a complication of dental injury (6). The treatment aimed to eradicate the implicated bacteria. In most of these infections, the bacteria belong to the oropharyngeal flora. The existence of a dissemination risk and focal infection is a potential danger to the human body, and especially heart, joints, and other vital organs can be affected (7). Although these infections are mixed polymicrobial and the predominance of anaerobic bacteria unanimously agrees with the authors (8,9), the microbial virulence is characteristic of severe infection with at least the presence of a multi-resistant bacteria (10). These infections remain dreadful in their evolution and prognosis that can sometimes be life-threatening (11).

In Burkina Faso, very few studies to our knowledge have been conducted on the resistance profile of bacteria involved in apical periodontitis and cellulitis of endodontic origin. It is in this perspective that it appeared appropriate to perform the present study to know the implication in endodontic infections of G. sanguinis, an emerging pathogen, and to understand its profile of antimicrobial resistance.

Materials and Methods

Site, Period, and Type of Study

This was a prospective study conducted over a period of 5 months from June 2014 to October 2014. The collection of samples (exudate) was performed at the Municipal Center of Oral Health of Ouagadougou, and the microbiological analyses were regularly conducted at the Laboratory of Molecular Biology, Epidemiology and Surveillance of Bacteria and Viruses Transmitted by Food at Joseph KI-ZERBO University.

Diagnostic Criteria for Apical Periodontitis and Cellulitis of Endodontic Origin

Three key criteria were used to make the diagnosis of apical periodontitis: the existence of an endodontic bacterial contamination pathway, a negative response to pulp vitality tests, and the presence of spontaneous pain and exacerbated on percussion. The presence of a periapical radiolucency was also considered. As for cellulitis of endodontic origin, the diagnosis remains based on extraoral and intraoral examinations. The extraoral examination was conducted by examination of maxillary and/or cervicofacial swelling, skin inflammation, trismus, and sub-angulo-maxillary lymphadenopathy. The intraoral examination was conducted by searching of endodontic origin.

Inclusion and Exclusion Criteria

Only patients with apical periodontitis or cellulitis of endodontic origin were included. A medical history with human immunodeficiency virus, diabetes, cancer; corticosteroid therapy was an exclusion criterion. Patients who started antibiotic therapy only on the day of collection were included in the study. Cellulitis fistula to the skin or oral mucosa was excluded from the study. Apical periodontitis of the teeth with the open pulp chamber throught the oral cavity was also excluded to avoid probable exogenous bacterial contamination.

Data and Sample Collection

Data were collected using a form containing civil information, medical history, and eating habits. Oral hygiene was assessed using the retention index of Björby and Löe (12). After clinical diagnosis, sampling was conducted according to the method of Rôcas and Siqueira (13). For cellulitis samples, each patient rinsed the oral cavity for a few seconds with a 0.12% chlorhexidine solution before sampling. The swollen mucosa was sanitized with a 2% chlorhexidine solution. Then, using a sterile mounted syringe, 2 ml of the purulent exudate was aspirated. Apical periodontitis samples were obtained from the root canal. A sterile rubber dam was previously placed. Sterile, unitary paper point sized to fit the canal space was used for sampling after the tooth was accessed with a sterile bur. The collected exudate was immediately transferred to a sterile tube containing resazurin thioglycolate broth (Liofilchem, Italy). The tubes were conditioned at 4 °C in a cooler and then transported to the laboratory for microbiological analyses within 2 h.

Characterization of G. Sanguinis

From the anaerobic transport broth, a 10-µl aliquot of thioglycolate with resazurin (Liofilchem) was inoculated on Columbia agar (Liofilchem) supplemented with hemoglobin (Liofilchem) (14). Petri dishes were incubated at 37°C for 48–72 h in a jar containing Genbox (Liofilchem) to create partial anaerobiosis. Probable *Globicatella* colonies (small colonies of 0.5–1 µm, short chain, α hemolytic, catalase negative, and hydrogen sulfide negative) were subcultured on Mueller-Hinton medium (Liofilchem) for

biochemical confirmation with the API 20 STREP gallery (bioMérieux, France). The reading was made according to the manufacturer's recommendations and then interpreted with the Apiweb software version V7.0.

Antimicrobial Susceptibility Testing

A 0.5 McFarland inoculum was used to perform the antibiogram using the disk diffusion method in agar medium (15). The diameters of the sensitivity of the antibiotic disks were read according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (ACFSM, 2017) [16]. Twenty-one antibiotics were used: oxacillin (5 µg), amoxicillin (30 µg), amoxicillin–clavulanic acid (20 + 10 µg), cefotaxime (30 µg), cefuroxime (30 µg), cefixime (5 µg), ceftriaxone (30 µg), erythromycin (15 µg), trimethoprim–sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), gentamicin (10 µg), tobramycin (10 mcg), netilmicin (30 µg), piperacillin (100 µg), piperacillin–tazobactam (100 + 10 µg), metronidazole (5 µg), penicillin G (10 IU), lincomycin (15 µg), and spiramycin (100 µg). The zones of inhibition were classified as "resistant," "intermediate," and "sensible" (16).

Phenotypic Detection of Extended Spectrum β-lactamases

Resistant β-lactam strains were subjected to the extended spectrum β-lactamases (ESBLs) synergetic test according to ACFSM recommendations (ACFSM, 2017) (16).

Statistical Analysis

Statistical analyses were done using the Sphinx Plus² version 5. χ^2 test was used for comparison of two qualitative variables. A p value < 0.05 was considered significant.

Results

Socio-Economic Characteristics of Patients

One hundred twenty-five subjects were examined including 62 (49.6%) males and 63 (50.4%) females (p = 0.9287) (Table 1). The age group of 19–40 years was the most represented (55.2%) (p = 0.0001). Low-income patients (farmers, students, pupils, and housewives; 47.2%) were the most affected (p = 0.0001). Patients with modest income (public employees, informal sector workers, retirees, and other sectors) accounted for 27.2% and high-income (private employees) for 25.6%.

Clinical Data

Sixty-two (49.6%) patients had cellulitis of endodontic origin, and 63 (50.4%) had apical periodontitis. Cellulitis accounted for 41.6% and apical periodontitis 32.8% at the acute stage. The difference was significant between the two infectious stages (p = 0.0001) (Table 2). Bad oral hygiene was 84.8% to which score 3 was assigned (p = 0.0001).

Distribution of Isolates

Five (4%) samples were positive to *Globicatella*. *G. sanguinis* is the only species that has been isolated. Three acute cellulitis samples, one of an apical periodontitis and one of a chronic cellulitis of endodontic origin, have been cause for concern. Some other bacteria have been identified in the three samples. They were *Staphylococcus lentus* in the first sample, *Aerococcus viridans* and *Aerococcus urinae* in the second sample, and *Staphylococcus xylosus* for the third sample.

Table 1. Distribution of endodontic infections by age group and gender

Gender	Age group (year)						Total, n (%)	p-value
	0–6	7–12	13–18	19–40	41–60	>60		
Male	1(0.8)	3 (2.4)	5 (4)	33 (26.4)	15 (12)	5 (4)	62 (49.6)	0.9287
Female	0 (0)	3 (2.4)	14 (11.2)	36 (28.8)	7 (5.6)	3 (2.4)	63 (50.4)	
Total, n (%)	1 (0.8)	6 (4.8)	19 (15.2)	69 (55.2)	22 (17.6)	8 (6.4)	125 (100)	

Table 2. Distribution of cellulitis and apical periodontitis by infectious stage

Pathology	Cellulitis		Apical periodontitis		Total, n (%)	p-value
	Acute	Chronic	Acute	Chronic		
Cellulitis	52 (41.6)	10 (8)	0 (0)	0 (0)	62 (49.6)	0.0001
Apical periodontitis	0 (0)	0 (0)	41 (32.8)	22 (17.6)	63 (50.4)	
Total, n (%)	52 (41.6)	10 (8)	41 (32.8)	22 (17.6)	125 (100)	

Table 3. Antimicrobial susceptibility of *Globicatella sanguinis*

Antibiotics	Susceptibility of isolates, n (%)		
	Resistant	Intermediate	Sensible
Amoxicillin-clavulanic acid	0 (0)	0 (0)	5 (100)
Ceftriaxone	2 (40)	3 (60)	0 (0)
Cefixime	3 (60)	0 (0)	2 (40)
Cefuroxime	4 (80)	0 (0)	1 (20)
Cefotaxime	5 (100)	0 (0)	0 (0)
Gentamicin	1 (20)	0 (0)	4 (80)
Clindamycin	2 (40)	0 (0)	3 (60)
Metronidazole	5 (100)	0 (0)	0 (0)
Tazobactam-piperacillin	0 (0)	0 (0)	5 (100)
Oxacillin	3 (60)	0 (0)	2 (40)
Spiramycin	1 (20)	3 (60)	1 (20)
Lincomycin	3 (60)	1 (20)	1 (20)
Piperacillin	1 (20)	0 (0)	4 (80)
Tobramycin	1 (20)	0 (0)	4 (80)
Netilmicin	0 (0)	2 (40)	3 (60)
Erythromycin	1 (20)	1 (20)	3 (60)
Trimethoprim-sulfamethoxazole	0 (0)	0 (0)	5 (100)
Chloramphenicol	1 (20)	0 (0)	4 (80)
Ciprofloxacin	2 (40)	0 (0)	3 (60)
Penicillin G	5 (100)	0 (0)	0 (0)
Amoxicillin	2 (40)	0 (0)	3 (60)

Antibiotic susceptibility testing

The strains of *Globicatella* isolated were resistant (100%) to cefotaxime, metronidazole, and penicillin G. Spiramycin presented an intermediate sensitivity of 60%. By contrast, the isolates showed a good sensitivity (100%) to trimethoprim–sulfamethoxazole, amoxicillin–clavulanic acid (Fig. 1), and tazobactam–piperacillin (Table 3). One of the five strains produced extended spectrum ß-lactamases (ESBLs) (Fig. 2).

Discussion

This study used the standard phenotypic methods to identify isolates and showed that this emerging bacterium can be found in endodontic infections. Male, low-income, and young patients were the most represented. Previous studies had reported the same trends (17–19). Antibiotics are routinely prescribed by dental surgeons to treat and prevent infections and reduce postoperative pain (20). The surgical procedure is often sufficient to treat the pathology. However, unfortunately, this over-prescription contributes to the development of the antibiotic resistance phenomena (20). This also concerns self-medication, which is highly exploited particularly in patients with low socio-economic level (19). African countries face many developmental challenges including oral health problems (21). Endodontic infections are caused by a multi-species bacteria, usually organized as biofilms adhering to the root canal walls (22). The ideal issue of treatment is to sterilize the root canal space to regain physiological conditions (23). Root canal disinfection and obturation only allow the eradication of infection at the apical level (24). *G. sanguinis* is an unusual pathogenic agent. However, it is an opportunistic bacterium that can be responsible for severe infections such as gastroenteritis, osteomyelitis, endocarditis, meningitis, and urinary tract infection (25,26). It can be misidentified as *Streptococcus pneumoniae* or *viridans* or *Aerococcus* due to their morphological resemblance (3). The main characteristic of differentiation between *Globicatella* and *Aerococcus* is the arrangement of the cells in Gram coloring. *Globicatella* is shaped like

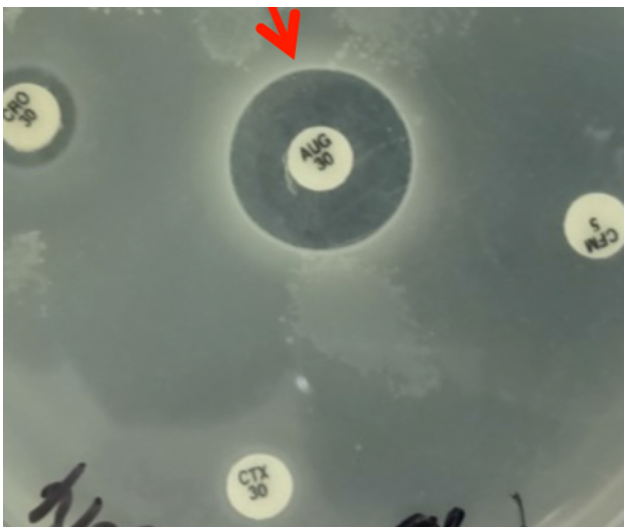

Fig. 1. Large sensitivity of *Globicatella sanguinis* to amoxicillin–clavulanic acid disc.

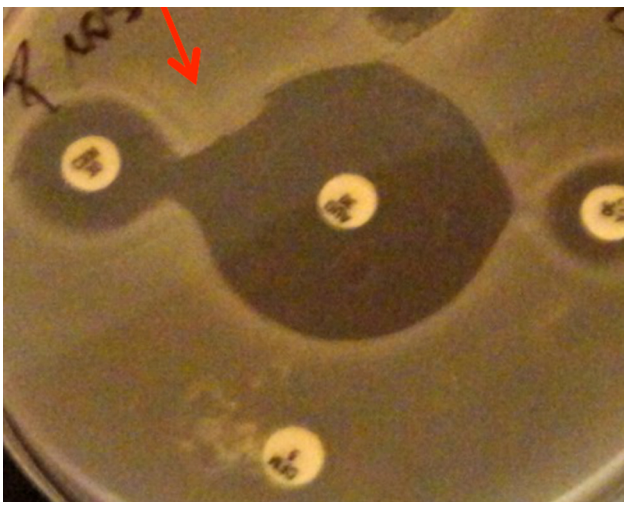

Fig. 2. The presence of extended spectrum β-lactamases is indicated by an effect between antibiotic disks, producing an extended halo with the appearance of a "champagne cork".

chains, whereas *Aerococcus* forms tetrads and clusters. The morphology of the colony of *Globicatella* strains is similar to *S. viridans*. In our study, isolated strains were resistant (100%) to cefotaxime, metronidazole, and penicillin *G. Spiramycin* presented an intermediate sensitivity of 60%. By contrast, the isolates showed good sensitivity (100%) to trimethoprim–sulfamethoxazole, amoxicillin–clavulanic acid, and tazobactam–piperacillin. In the absence of an interpretive directive approved by the Clinical and Laboratory Standards Institute for *Globicatella*, it is recommended to use the minimum inhibitory concentration (2). However, in the reported cases of *Globicatella* infections, the antibiotic sensitivity results were found on the basis of the criteria of the *S. viridan*s group (2,26). Shewmaker et al. (27) reported that the rates of *G. sanguinis* antibiotic sensitivity to antibiotic resistance of cefuroxime, cefotaxime, and meropenem are 74%, 48%, and 37%, respectively. The authors reported, in contrast to our results, a sensitivity of 100% to penicillin, amoxicillin, cefotaxime, ceftriaxone, meropenem, erythromycin, and levofloxacin. Our results have shown that macrolides, quinolones, and third-generation cephalosporins have presented a fairly high strength. Many other studies also report resistance to third-generation cephalosporins (25,26,28). By contrast, Matulionyté et al. (25) reported resistance to trimethoprim–sulfamethoxazole. A strain has been found to produce ESBLs. These resistances could be explained by the presence of virulence genes, which can be transferred to other bacteria in biofilm (29–31). Although the reporting of a *G. sanguinis* infection has been rare, the bacterium was related to severe infections (26). These cases of infections unfortunately concerned elderly people (2,28). It is a bacterium rarely isolated from clinical samples (25). *G. sanguinis* infects farm animals and the breeder, but the contamination pathway was unknown (28). Strains of *G. sanguinis* have been identified in the oral cavity and the flesh of tilapia (32), a fish that is consumed in Burkina Faso. This consumption could explain contamination. Maryam et al. (33) reported that air and water in restaurants open to the public are contaminated by G. sanguinis. Héry-Arnaud et al. (28) have identified it from a patient with meningitis who was vomiting. The epidemiology and clinical signs of this pathogen remain largely unknown. Some studies report the presence of *Globicatella* in oral infections. This is probably due to confusion in its identification with other bacterial genera. The emergence of *G. sanguinis* resistant to multiple antibiotics including β-lactams, which are among the most prescribed molecules, could become a public health concern. The antibiotic sensitivity profile of the five strains studied has shown resistance to most β-lactams and macrolides that are common prescriptions in dentistry. The oral cavity could be the starting point for bacterial dissemination to some other organs (34). Dental infection may provoke serious consequences. Infection, cellulitis, and abscess of dental origin should never be underestimated (35).

Conclusion

In Burkina Faso, a large proportion of oral diseases are not treated, and oral health care needs are high. Pathologies that can easily be supported at their initial stages are not. Thus, the infectious complications (apical periodontitis and cellulitis) often impose the use of antibiotics. The treatment of endodontic infections requires the disinfection of the root canal space with the interest to know the bacteria involved. The emergence of *G. sanguinis* and the diffusion of multi-resistant antibiotic bacteria can lead to great difficulties of care, with situations of therapeutic impasse.

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Ethical Approval: This study protocol has been approved by the national ethics committee of Burkina Faso (Deliberation No. 2009-30 issued on July 17, 2009).

Informed consent: Written informed consent was obtained from patients who participated in this study.

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