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Occurrence and antimicrobial susceptibility of *Leuconostoc*: An emergent pathogenic associated with acute endodontic infections

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Purpose: Endodontic infections are polymicrobial. This study was undertaken to characterize and perform an antimicrobial susceptibility test on *Leuconostoc* sp. isolated from acute endodontic infections in Ouagadougou, Burkina Faso.

Methods: A cross-sectional descriptive study was conducted at Ouagadougou, Burkina Faso, from June to October 2014. A questionnaire form was used to collect the clinical data. Biochemical standard techniques were used to isolate bacteria, and identification was made by API 20 Strep gallery. Antimicrobial susceptibility was carried out by the diffusion method on a solid medium.

Results: The age group of 19–40 years (67.7%) was more significant among the 93 patients included in the study. Acute cellulitis was accounted for 66.7% while acute apical periodontitis was observed in 33.3% of cases. Two exudate samples were positive to *Leuconostoc* sp. showing 100% sensitivity to lincosamides (lincomycin and clindamycin). On the other hand, they were 100% resistant to trimethoprim-sulfamethoxazole, 50% resistant to the macrolides (spiramycin and erythromycin), and the 3rd generations of cephalosporins (cefotaxime, cefuroxime, cefixime, and ceftriaxone).

Conclusion: *Leuconostoc* sp. was associated with acute endodontic infections and an opportunistic pathogenic bacterium. The primary objective of the treatment of endodontic infection is to eliminate biofilms by a chemomechanical treatment. Dentists must avoid the overuse of antibiotics to prevent the emergence of antibiotic-resistant bacterial strains.

Keywords: Acute endodontic infections, Antimicrobial susceptibility, *Leuconostoc* sp.

Introduction

The common cause of dental infections is pulp necrosis, which is followed by bacterial invasion through the pulp chamber into the deeper tissues. Several studies (1,2) have reported on the microbial composition of necrotic dental pulps, the presence of facultative anaerobic and obligate anaerobic bacteria, which are usually present in oral environment. Bacteria in the root canal may progress and their products may spread to the periapical tissues. This study focused on *Leuconostoc* sp. which is an emerging bacterium

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in human pathology. *Leuconostoc* is a Gram-positive ovoid coccus that is intrinsically vancomycin resistant and is typically non-pathogenic to humans (3). However, some recent studies (4–6) reported that *Leuconostoc* species caused infective endocarditis of the aortic valve. These cases serve to remind clinicians that *Leuconostoc* species may cause infective endocarditis in individuals with a history of intravenous drug use or a history of dental abscess (4,7). The importance of maintaining good oral health for systemic health is an ancient concept but just as relevant today. Direct or indirect mechanisms have been proposed for links between oral bacteria and systemic diseases (8). *Leuconostoc mesenteroides, Leuconostoc pseudomesenteroides, Leuconostoc lactis, Leuconostoc argentinum, Leuconostoc citreum, Leuconostoc fallax, Leuconostoc gelidum,* and *Leuconostoc carnosum* are the eight validly described species (9). To the best of our knowledge, no study has been undertaken on the implication of *Leuconostoc* sp. in endodontic infections in Burkina Faso. The aim of this prospective study was to investigate *Leuconostoc* strains in samples taken from root canals of deciduous and permanent teeth with acute endodontic infections and to determine the antimicrobial susceptibility for each isolate.

Materials and Methods

Study Area, Period, and Settings

It was a cross-sectional descriptive study performed in Ouagadougou, Burkina Faso. Exudate samples were collected from June to October 2014.

Inclusion and Exclusion Criteria

Patients with acute apical periodontitis or acute cellulitis of endodontic origin were included in the present study. The patients were all examined by a dental surgeon and those who began an antibiotherapy on the day of sampling were included. Both permanent or deciduous teeth in any patient were considered.

Criteria for exclusion were as follows:

- Any tooth that has been previously root canal filled
- The presence of a periodontal pocket of 5 mm or more
- The presence of a fistula or the pulp chamber exposed to oral cavity.

No medical history has been an exclusion criterion.

Data Collection Process

Data were collected using a form. Demographic information (age, gender, etc.), medical history, and professional data were compiled. Sampling was carried out according to the method of Rôcas and Siqueira (10). Exudate samples were transferred to a sterile tube containing resazurin thioglycolate broth (Liofilchem, Italy). Tubes were refrigerated at 4°C in a cooler and transported immediately to the laboratory for microbiological analysis within 2 following hours.

Isolation and Identification of Leuconostoc spp.

An aliquot (10 µl) of the anaerobic transport broth, thioglycolate with resazurin (Liofilchem, Roseto degli Abruzzi, Italy), was inoculated on Columbia agar (Liofilchem, Roseto degli Abruzzi, Italy) supplemented with hemoglobin (Liofilchem, Roseto degli Abruzzi, Italy) (11). Petri dishes were placed in a jar with Genbox (Liofilchem, Roseto degli Abruzzi, Italy) and then incubated at 25°C for 48–72 h. The probable colonies of *Leuconostoc* spp. (cocci in chains or pairs, white, raised, and small, catalase negative, and oxidase negative) were subcultured on Mueller-Hinton medium (Liofilchem, Roseto degli Abruzzi, Italy). Biochemical characterization was confirmed using API 20 STREP gallery (bioMérieux, Marcy-l'Étoile, France) and the interpretation was done with the Apiweb software version V7.0.

Antimicrobial Susceptibility Testing

Disk diffusion susceptibility testing of the isolates to 21 antimicrobial agents (Table 1) was performed (12). Antibiotic

Table 1. Antibiotics tested to isolates

Antibiotics families	Antibiotics	Concentration
ß-lactams		
Penicillins	Amoxicillin-clavulanic acid	20+10 µg
	Amoxicillin	30 µg
	Penicillin G	10 IU
	Piperacillin	100 µg
	Piperacillin-tazobactam	100+10 µg
	Oxacillin	5 µg
Cephalosporins	Ceftriaxone	30 µg
	Cefixime	30 µg
	Cefuroxime	30 µg
	Cefotaxime	30 µg
Phenicols	Chloramphenicol	30 µg
Quinolones	Ciprofloxacin	5 µg
Sulfamides	Trimethoprim-sulfamethoxazole	1.25/23.75 µg
Nitro-imidazols	Metronidazole	5 µg
Aminosids	Gentamycin	10 µg
	Tobramycin	10 µg
	Netilmicin	30 µg
Macrolides	Erythromycin	15 µg
	Spiramycin	100 µg
Lincosamides	Clindamycin	10 µg
	Lincomycin	15 µg

Table 2. Food habits of patients

Answer	Rates of food consumed by patients, n (%)				
	Meat products	Dairy products	Fish products	Sweet products	Fruits/vegetables
Yes	35 (37.6)	15 (16.1)	29 (31.2)	9 (9.7)	4 (4.3)
No	58 (62.4)	78 (83.9)	64 (68.8)	84 (90.3)	89 (95.7)

disks were placed on plates that were incubated at 37°C for 48–72 h. The diameters of the antibiotic sensitivity halos were recorded according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017) (13). Intermediary (I) susceptibility of pathovars was classified as resistant (R).

Phenotypic Detection of Extended Spectrum ß-Lactamase (ESBL)

An investigation of extended spectrum ß-lactamase was carried out for strains that were β-lactams resistant according to the recommendations of EUCAST (2017) (13). The synergetic test was performed between three antibiotic disks (amoxicillin-clavulanic acid, ceftriaxone, and cefotaxime) placed on the bacterial plate and separated by 2–3 cm from one another. ESBL presence is indicated by an effect between disks, giving rise to an extended halo with appearance of a "champagne cork" of keyhole.

Statistical Analysis

The χ^2 (Chi-square) test of the Sphinx V.5 software (Parc Altaïs, Chavanod, France) was used to analyze the data collected. P< .05 was considered statistically significant.

Ethical Approval and Consent to Participate

This research has been approved by the national ethics committee of Burkina Faso (Deliberation N 2009-30 issued on July 17, 2009). All the data were obtained with informed patient consent.

Results

Socioeconomic Characteristics of Patients

Ninety-three patients were included, consisting of 39 males (41.9%) and 54 females (58.1%). There are a fairly high number of cases in patients aged between 19 and 40 years (67.7%) (p< 0.001). Acute cellulitis was present in 66.7% and apical periodontitis in 33.3% of cases. Patients with low income (50 [53.8%]) were found to be the most affected (p< 0.001). Poor oral hygiene was determined in 76 (81.7%) patients (p< 0.001). Meat products (37.6%) and fish products (smoked fish) (31.2%) were mostly consumed by patients (Table 2).

Table 3. Antimicrobial susceptibility of *Leuconostoc* spp.

Antibiotics	Susceptibility of isolates, n (%)	
	Resistant (R+I)	Sensitive
Amoxicillin-clavulanic acid	0 (0)	2 (100)
Ceftriaxone	1 (50)	1 (50)
Cefixime	1 (50)	1 (50)
Cefuroxime	1 (50))	1 (50)
Cefotaxime	1 (50)	1 (50)
Gentamycin	0 (0)	2 (100)
Clindamycin	0 (0)	2 (100)
Metronidazole	2 (100)	0 (0)
Piperacillin-tazobactam	0 (0)	2 (100)
Oxacillin	1 (50))	1 (50)
Spiramycin	1 (50))	1 (50)
Lincomycin	0 (0)	2 (100)
Piperacillin	0 (0)	2 (100)
Tobramycin	0 (0)	2 (100)
Netilmicin	1 (50)	1 (50)
Erythromycin	1 (50)	1 (50)
Trimethoprim-sulfamethoxazole	2 (100)	0 (0)
Chloramphenicol	1 (50)	1 (50)
Ciprofloxacin	1 (50)	1 (50)
Penicillin G	0 (0)	2 (100)
Amoxicillin	1 (50)	1 (50)

Bacterial Etiologies and their Antibiotic Susceptibility Profile

Among the 93 exudate samples, 2 (1.6%) were positive for *Leuconostoc* spp. that have been isolated from one acute facial cellulitis and an acute apical periodontitis. *Leuconostoc* isolates showed 100% sensitivity to lincosamides (lincomycin and clindamycin). The beta-lactam antibiotics to which isolates were 100% sensitive are piperacillin, tobramycin, amoxicillin-clavulanic acid, gentamycin, piperacillin-tazobactam, and penicillin G. By contrast, isolates were 100% resistant to trimethoprim-sulfamethoxazole. Sensitivity was 50% for macrolides (spiramycin and erythromycin) and for the 3rd generation cephalosporins antibiotics (cefotaxime, cefuroxime, cefixime, and ceftriaxone) (Table 3).

Discussion

Overconsumption of antibiotics resulting in the emergence of antibiotic-resistant bacterial strains is a global concern (14). Dental surgeons may overuse antibiotics to

treat oral infections while it is not always indicated. These infections are generally due to oral microbiota (14). *Leuconostoc* species are Gram-positive cocci belonging to the *Leuconostocaceae* family. Species were generally considered non-pathogenic and of little significance in clinical microbiology until several occurrences of *Leuconostoc* infections were reported in the literature. It is an emerging pathogen responsible for many infections (15,16). Standard phenotypic methods were used in this research to identify isolates. Indeed, *Leuconostoc* may be misidentified by routine biochemical testing. Facklam et al. (17) reported that *Leuconostoc* is often misidentified as *Lactobacillus, Streptococcus, Pediococcus, Enterococcus,* or *Lactococcus*. This misidentification can lead to an underestimation of the true incidence rate. Notwithstanding this, the present study showed that *Leuconostoc* sp. may be involved in root canal infections. Some studies (1,18) isolated *Leuconostoc* sp. in endodontic infections. These studies reported a prevalence of 1.2% and 2.9%. A previous study (19) also reported an odontogenic infection caused by *Leuconostoc* sp.

Antibacterial therapy is one of the most important medical developments of the past century. Unfortunately, the development of antimicrobial resistance threatens antibiotic therapy (20). An excessive use and a misuse of antibiotics increase the selective pressure favoring the emergence, multiplication, and spread of resistant strains. Dental surgeons currently use broad-spectrum antibiotics. In most cases, the prescription of antibiotics in endodontic infections is empirical and an overuse is observed (21). This contributes to the emergence of antibiotic-resistant bacterial strains (14). In this study, the isolated strains were 100% resistant to trimethoprim-sulfamethoxazole. By contrast, they were 100% sensitive to lincosamides (lincomycin and clindamycin). Beta-lactam antibiotics which were 100% sensitive are piperacillin, tobramycin, amoxicillin-clavulanic acid, gentamycin, piperacillin-tazobactam, and penicillin G. Sensitivity was intermediate for macrolides (spiramycin and erythromycin) and 3rd generation cephalosporins antibiotics. The study of Wenocur et al. (19) reported as in this work that the isolate was susceptible to penicillin. Unlike many Gram-positive bacteria, *Leuconostoc* species commonly demonstrate high-level resistance to vancomycin, with preserved sensitivity to most other antibacterial agents (22). A recent study (15) reported a case of *Leuconostoc* acute endophthalmitis. The authors showed that *Leuconostoc* was sensitive to ceftazidime, gentamicin, ciprofloxacin, amikacin, gatifloxacin, and moxifloxacin. As of today, the source of the contamination is not really known. *Leuconostoc* species exist in fermented foods and beverages industries due to their role in fermentation (sauerkraut, milk, cereals fermented drinks, and palm wine) (23). This bacterium is found naturally in plants and vegetables (16). Humans may be infected by exposure to unpasteurized products or by contact with vegetables or plants (24). Some studies (25,26) in Burkina Faso show a significant consumption of palm wine and milk. This consumption could explain the contamination. In addition, these studies (25,26) report uncontrolled use of antibiotics in livestock systems. Tapsoba et al. (26) detected the presence of *Leuconostoc* sp. in palm wine in Burkina Faso. The main dangers to the consumer are the presence of antibiotic residues and bacteria resistant to antibiotics (27). Although they are resistant to vancomycin, *Leuconostoc* are sensitive to erythromycin and clindamycin (16). A recent study (28) reported *Leuconostoc* endocarditis and suggested poor dental health or odontogenic infections to be the major source. *Leuconostoc* sp. have only recently been recognized as potential pathogens. The antibiotic susceptibility study of the two isolated strains revealed resistance to most of the β-lactams and macrolides. These families of antibiotics are common in odontology (14). The most prescribed antibiotics in dental practice are β-lactam antibiotics and penicillin (2,29). Antibiotic therapy may be necessary to prevent a risk of spread of the infection in acute apical abscesses with possible systemic involvement (12). Microbial biofilms that adhere to the root canal dentine can extend to the apical foramina and sometimes beyond. The objectives of root canal treatment are to eliminate these biofilms using chemomechanical treatment protocols and to prevent reinfection (30).

Conclusion

Dental infection is polymicrobial and *Leuconostoc* sp. may be implicated. This infection is a common and potentially life-threatening condition in some areas where admissions for surgical treatment of endodontic infections are increasing. The presence of at least one multidrug-resistant bacterium can compromise successful treatment. Antibiotics are not appropriate in the absence of signs of spreading infection or systemic upset. The use of systemic antibiotics in endodontics should be limited to specific cases to avoid their overprescription. In this study, *Leuconostoc* isolates showed 100% sensitivity to lincomycin, clindamycin, piperacillin, tobramycin, amoxicillin-clavulanic acid, gentamycin, piperacillin-tazobactam, and penicillin G.

Data Availability Statement

The data used to support the findings of this study are currently non available while their exploitation is continuing. Requests for data 12 months after publication of this manuscript will be considered by the corresponding author.

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