

## ANTIBIOTIC SUSCEPTIBILITY TESTING OF SOME *PREVOTELLA* STRAINS ISOLATED FROM VESTIBULAR ABSCESSSES

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### Abstract

The aim of this study was to investigate *in vitro* the antibiotic susceptibility of 53 strains of *Prevotella* isolated from vestibular abscesses. The E-test was used for determining the minimum inhibitory concentrations for: benzylpenicillin, ampicillin, amoxicillin/clavulanic acid, clindamycin and metronidazole. In addition to this, the beta-lactamase production was detected by the chromogenic cephalosporin test. Most of the strains were susceptible to the tested antibiotics. Twelve strains were resistant to benzylpenicillin and ampicillin due to the production of beta-lactamase. A single isolate of *P. buccae* presented also resistance to clindamycin. In conclusion, the results of this study recommend the prescription of metronidazole or clindamycin in infections caused by these microorganisms. In case of oral and maxillofacial infections (which are mainly mixed infections), when antimicrobial treatment is necessary but it is done empirically, it might be proper to choose an antibiotic active against beta-lactamase producing bacteria too, such as an association of a penicillin with a beta-lactamase inhibitor.

### Rezumat

Acest studiu a avut drept scop investigarea *in vitro* a sensibilității la antibiotice a 53 de tulpini de *Prevotella* izolate din abcese vestibulare. A fost utilizat *E-test*-ul pentru determinarea concentrațiilor minime inhibitorii pentru: benzilpenicilină, ampicilină, amoxicilină/acid clavulanic, clindamicină și metronidazol. Pe lângă acest aspect, a fost detectată și producerea de beta-lactamază prin testul cu o cefalosporină cromogenă. Majoritatea tulpinilor au fost sensibile la toate cele 5 antibiotice. Un număr de 12 tulpini s-au dovedit a fi rezistente la benzilpenicilină și ampicilină, datorită elaborării de beta-lactamază. Un singur izolat de *P. buccae* a prezentat concomitent șirezistență la clindamicină. În concluzie, rezultatele acestui studiu local recomandă utilizarea în infecțiile produse de aceste microorganisme a metronidazolului sau a clindamicinei. În cazul infecțiilor orale și maxilo-faciale (ce sunt cu precădere infecții mixte), atunci când tratamentul antimicrobian se impune, însă este practicat empiric, ar fi adecvată alegerea unui antibiotic activ inclusiv pe bacteriile producătoare de beta-lactamază, precum asocierea unei peniciline cu un inhibitor de beta-lactamază.

**Keywords:** oral infections, *Prevotella*, antibiotic resistance

### Introduction

The oral infections are mainly mixed infections, involving usually both aerobic and strictly anaerobic microorganisms, with the last ones predominating [15]. The most frequently anaerobic bacteria associated with infections of dental origin are represented by the Gram-negative bacilli, such as different species of *Prevotella*, *Porphyromonas*, *Bacteroides*, *Tannerella* and *Fusobacterium*. Most oral and maxillofacial abscesses require only surgical incision and drainage as treatment. However, the antibiotics are needed sometimes in order to prevent the local or distant spread of the infection, especially in immuno-compromised patients. Based partially on the fact that the anaerobic bacteria are usually fastidious and slow-growing, many physicians consider the isolation and identification of the strictly anaerobes as time

consuming and prefer to prescribe empirically the antibiotics in this type of infections.

Penicillin is still considered to be the antimicrobial agent of choice for the treatment of oral infections, but there are a lot of recent studies which reported penicillin resistant anaerobic strains [5, 13, 16]. In Romania, there are only few published data concerning the identification at species level of the anaerobic isolates with clinical importance and their susceptibility to commonly used antibiotics. Since the vestibular abscess is considered to be the most frequent odontogenic infection, the aim of this study was to investigate the *in vitro* antibiotic susceptibility of *Prevotella* isolates from Romanian patients diagnosed with this type of infection.

### Materials and Methods

In this study there were used 53 strains of *Prevotella* from a collection of clinical isolates

belonging to the laboratory of the Discipline of Microbiology, Faculty of Dentistry, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania. These strains were isolated from 50 pus samples collected by needle aspiration from patients with vestibular abscess who presented to the Dental University Hospital – Bucharest, Romania, and to the Private Dental Practice "Dr. Mircea Pănculescu", between January 2008 - January 2011. The respective strains were identified at genus level by conventional methods and were stored on cryo-bead tubes (AES Laboratoire, France), at -70°C, in the laboratory mentioned above.

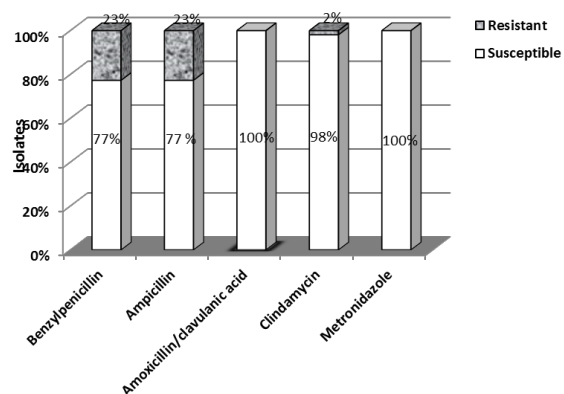
In the present study, the 53 non-duplicate strains, including also 3 pairs of *Prevotella* isolates originated from 3 patients with vestibular abscess, were identified at species level using the Rapid ID 32 A system (BioMérieux, Marcy-l'Etoile, France).

All strains were tested for their susceptibility to: benzylpenicillin, ampicillin, amoxicillin/clavulanic acid, clindamycin and metronidazole by the E-test (BioMérieux, Marcy-l'Etoile, France). The reference strains *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were used for quality control. An inoculum in Schaedler broth with vitamin K<sub>3</sub> (BioMérieux, Marcy-l'Etoile, France), adjusted to the turbidity of 1 McFarland, was prepared for each strain and swabbed onto *Brucella* E-test agar plates (AES Laboratoire, France). After the E-test strips were displayed on the inoculated agar surface, the plates were incubated in jars with anaerobic atmosphere generated by using the *Genboxanaer* envelopes (BioMérieux, Marcy-l'Etoile, France), at 35°C for 48 - 72 h. The minimum inhibitory concentrations (MICs) were read at the point of complete inhibition of the bacterial growth, after 48 h of incubation. In the case of metronidazole a second reading was performed at 72 h of incubation. The values of MICs were interpreted according to the recommendation of the Clinical and Laboratory Standards Institute (CLSI) [6]. In addition, the *Prevotella* strains were tested for beta-lactamase production by the chromogenic cephalosporin method, using nitrocefin impregnated sticks (Oxoid, UK) and *B. fragilis* ATCC 25285 as positive control.

## Results and Discussion

The *Prevotella* strains belonged to the following species: *P. melaninogenica* (18 isolates), *P. intermedia* (11 isolates), *P. oralis* (11 isolates), *P. denticola* (5 isolates), *P. buccae* (5 isolates) and *P. loscheii* (3 isolates). The strains belonging to the pairs of *Prevotella* isolates from the same vestibular abscess have been identified in all 3 cases as *P. intermedia* and *P. oralis*, respectively.

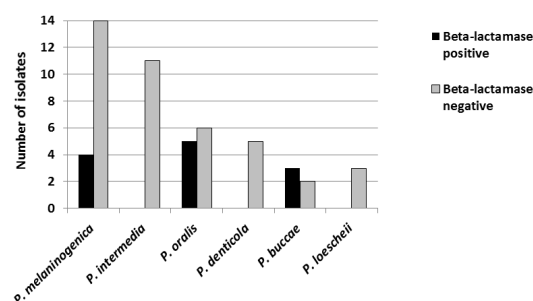
Figure 1 shows the distribution of the isolates according to the results of their susceptibility testing against the 5 antimicrobial agents. The MIC values which inhibited 50% (MIC<sub>50%</sub>) and 90% (MIC<sub>90%</sub>) of the strains were the following: 0.125 and 32 mg/L for benzylpenicillin, 0.094 and 12 mg/L for ampicillin, 0.094 and 0.5 mg/L for amoxicillin/clavulanic acid, 0.032 and 0.125 mg/L for clindamycin, and 0.064 and 0.19 mg/L for metronidazole.



**Figure 1.**

The percentage distribution of the isolates by their susceptibility tested against five antimicrobial agents

All isolates were susceptible to amoxicillin/clavulanic acid and metronidazole, with same MICs values found at 48 h and 72 h. Twelve strains were resistant to benzylpenicillin and ampicillin and they belonged to the following species: *P. oralis* (5 isolates, including the 3 strains isolated together with the *P. intermedia* isolate from the same abscess), *P. melaninogenica* (4 isolates) and *P. buccae* (3 isolates). One *P. buccae* strain was resistant to clindamycin too. The activity of beta-lactamases has been detected only in the strains which showed resistance to the beta-lactam antibiotics tested by E-test (Figure 2).



**Figure 2.**

The isolates distribution by species and beta-lactamase activity

Many studies reported *Prevotella* as being the most frequently anaerobic microorganism involved in the

etiopathogeny of the head and neck infections [5, 16, 17]. The main *Prevotella* species involved in the oral and maxillofacial infections is considered to be *P. intermedia*. However, *P. melaninogenica* predominated in this series of strains and it was followed by *P. intermedia* and *P. oralis*.

The *in vitro* susceptibility to antimicrobial agents is very seldom investigated for strains isolated from oral infections. Nevertheless, it is recommended to verify periodically the local resistance patterns of the isolates with clinical significance and the E-test is a very convenient method to be applied in case of the strictly anaerobic bacteria [11, 12]. In the present research work, the E-test conferred clear endpoints in all cases.

A lot of recent studies have indicated high rates of penicillin resistance among *Prevotella* strains isolated from oral or other infections [9, 10]. In this study, 23% of the isolates were resistant to both benzylpenicillin and ampicillin, but some researchers found much higher percentages (of 60 - 90%) [13]. In the case of *Prevotella*, the penicillin resistance is mostly due to the beta-lactamase activity, which usually represents the expression of *cfxA* and *cfxA2* genes [8]. The 12 isolates with MICs values indicating resistance to beta-lactam antibiotics were all positive for this enzyme activity. Other authors found much higher proportions of beta-lactamase producers (about 30 - 60%) among *Prevotella* isolates [4, 7]. In this scientific paper, the beta-lactamase producers belonged to 3 species, noticing that *P. intermedia* and *P. loescheii* isolates lack to produce this enzyme. Some authors observed that two thirds of the *P. melaninogenica* isolates were positive for beta-lactamase production [3].

A single *P. buccae* isolate showed a very high MIC value in case of clindamycin (exceeding the maximum antibiotic concentration indicated by the E-test strip), while the literature has indicated a frequency of *Prevotella* strains resistant to this antibiotic from 4% to 25% [3, 5].

Metronidazole was fully active against the 53 strains of *Prevotella*, with the same MICs values recorded at both readings. Resistance to this antibiotic was very seldom found among the anaerobic Gram-negative bacilli isolated from oral and maxillofacial infections [10]. The polymerase chain reaction method became a useful application for detecting different bacterial genes [1, 2], including the antibiotic resistance genes [15]. The 5-nitroimidazole (*nim*) resistance gene has been proved to be responsible for the inducible high level metronidazole resistance and it was detected also in some *Prevotella* strains [14].

## Conclusions

When antibiotics are needed in the treatment of odontogenic infections and this is done empirically, an association of penicillin with a beta-lactamase inhibitor should be preferred, due to the frequent presence of beta-lactamase producers in such infections. Clindamycin (like metronidazole) is very active on most anaerobes and might represent a proper alternative in Romanian patients allergic to beta-lactam antibiotics.

At present, Romanian data regarding the antimicrobial susceptibility of anaerobe isolates are very poor and further studies of larger samples are strongly recommended, due to the importance of these bacteria involved in many oral and other infections.

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