Antimicrobial susceptibility of *Prevotella* isolates from abscesses of fascial spaces of the face and neck

Susceptibilitatea antimicrobiana a izolatelor de *Prevotella* din abcese ale ale spatiului fascial al feței şi gîtului

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Abstract

The aim of the present study was to investigate the antimicrobial susceptibility of a collection of 65 Prevotella strains originating from Romanian patients with abscesses of fascial spaces of the face and neck. The minimum inhibitory concentrations of benzylpenicillin, cefoxitin, clindamycin and metronidazole were determined with the Etest. In addition, the beta-lactamase production was assessed by using a chromogenic cephalosporin disc method. The isolates were sensitive to all antibiotics, except for 13 strains which were resistant to benzylpenicillin, due to their beta-lactamase activity. The results of this local investigation on the susceptibility patterns of Prevotella clinical isolates suggest that the combination penicillin - beta-lactamase inhibitor, clindamycin and metronidazole should be recommended in infections produced by these bacteria. The findings of the present study also underline the importance of covering both aerobic and anaerobic bacteria, including beta-lactamase producers, with the antimicrobial treatment, when it is needed and prescribed empirically in the oral and maxillofacial infections, which are known to be mostly mixed infections.

Key words: *Prevotella, antimicrobial susceptibility, head and neck infections*

Rezumat

Scopul prezentului studiu a fost acela de a investiga sensibilitatea la antibiotice a unei colecții de 65 tulpini de Prevotella provenite de la pacienți români diagnosticați cu abcese localizate în lojile superficiale ale feței şi gâtului. Concentrațiile minime inhibitorii pentru: benzilpenicilină, cefoxitină, clindamicină şi metronidazol au fost determinate cu ajutorul Etest-ului. Totodată, a fost testată şi producerea de beta-lactamază prin metoda discului cu

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cefalosporină cromogenă. Izolatele au fost sensibile la toate antibioticele, cu excepţia a 13 tulpini rezistente la benzilpenicilină datorită acţiunii beta-lactamazei. Rezultatele acestei investigări la nivel local a pattern-urilor de sensibilitate în cazul izolatelor clinice de Prevotella sugerează că: asocierea dintre o penicilină si un inhibitor de beta-lactamază, clindamicina şi metronidazolul pot fi recomandate în infecţiile produse de aceste bacterii. De asemenea, constatările prezentului studiu subliniază oportunitatea administrării unor antibiotice active atât pe bacteriile aerobe, cât şi pe cele anaerobe, inclusiv pe cele producătoare de beta-lactamază, în situaţia în care tratamentul antimicrobian este necesar şi prescris empiric în infecţiile oro-maxilo-faciale, cunoscute a fi cu precădere infecţii mixte.

Cuvinte cheie: *Prevotella, sensibilitatea la antibiotice, infecţii cu localizare la nivelul capului şi gâtului*

Introduction

The genus *Prevotella* includes species of Gram-negative anaerobic bacilli that are frequently isolated from different human infections (1, 2), as *P. intermedia* or *P. nigrescens*, which are commonly associated with infections of dental origin (3). Extensive scientific evidence indicate that the microflora of the oral and maxillofacial infections is mainly polymicrobial, involving aerobic and anaerobic microorganisms (4). Although the surgical incision and drainage are of primary importance for the treatment of the majority of such infections, which include also the abscesses of fascial spaces of the face and neck, the antibiotics are sometimes required for preventing the local spread or hematogenous dissemination of infection, especially in immunocompromised subjects. Unfortunately, the antimicrobial agents are often prescribed empirically, due in part to the time consuming nature of anaerobes cultivation and other fastidious bacteria involved in the pathogenesis of these infections (5). Penicillin is still considered the antimicrobial agent of choice for the oral and maxillofacial infections. However, a lot of concern has been expressed in the recent years regarding the increased number of penicillin resistant anaerobic isolates (6, 7).

Information regarding the prevalence and antimicrobial susceptibility of the Gram-negative anaerobic bacilli involved in the etiology of oral and maxillofacial infections in Romanian patients is very limited. Therefore, the aim of the present study was to investigate the antibiotic resistance patterns of *Prevotella* strains isolated from Romanian subjects with abscesses of fascial spaces of the face and neck.

Materials and method

Bacterial strains

The organisms tested were represented by 65 non-duplicate *Prevotella* strains (31 strains of *P. melaninogenica*, 18 strains of *P. denticola*, 11 strains of *P. oralis* and 5 strains of *P. loscheii*) belonging to a temporary collection of clinical isolates of the Department of Microbiology, Faculty of Dental Medicine, University of Medicine and Pharmacy "Carol Davila", Bucharest. The strains had been recovered from 61 non-duplicate pus samples collected by needle aspiration from subjects with abscess of: submandibular space, buccal space, sublingual space and submental space, among the patients with abscesses of fascial spaces of the face and neck presented to the Bucharest Dental University Hospital and the Private Dental Practice "Dr. Mircea Panculescu", Bucharest, during January 2005 - January 2008. These strains had been previously identified to species level using the conventional methods and Rapid ID 32 A system (BioMérieux, Marcy-l'Etoile, France) at the laboratory of the same department (previously reported data).

This series of *Prevotella* strains included 4 *P. denticola* strains isolated from submandibular abscesses together with one *P. loescheii* strain in 3 cases and with one *P. oralis* strain in another case. The isolates were stored in Greave's medium at -70^{0}C until used in the present study.

Table 1. The MICs of the antibiotics tested against the 65 *Prevotella* isolates

Species	Antibiotics	MIC (µg/ml)		
		Range	*MIC_{50}	**MIC_{90}
P. melaninogenica	Benzylpenicillin	0.032-2	0.064	2
	Cefoxitin	0.5-4	1	4
	Clindamycin	0.016-0.125	0.023	0.064
	Metronidazole	0.016-1	0.064	0.19
P. denticola	Benzylpenicillin	0.032-0.064	0.032	0.064
	Cefoxitin	0.5-3	1	2
	Clindamycin	0.016-0.064	0.023	0.032
	Metronidazole	0.016-0.5	0.032	0.19
P. oralis	Benzylpenicillin	0.032-2	0.032	2
	Cefoxitin	0.5-4	2	3
	Clindamycin	0.016-0.047	0.016	0.023
	Metronidazole	0.016-0.125	0.016	0.064
P. loscheii	Benzylpenicillin	0.032-0.064	0.064	0.064
	Cefoxitin	0.5-2	1	1.5
	Clindamycin	0.016-0.032	0.023	0.032
	Metronidazole	0.016-0.032	0.032	0.032

**MIC value inhibiting 50% of the isolates; **MIC value inhibiting 90% of the isolates.*

Susceptibility testing

The *Prevotella* isolates were tested for their susceptibility to benzylpenicillin, clindamycin, cefoxitin and metronidazole with the E-test (AB Biodisk, Solna, Sweden). *Bacteroides fragilis* ATCC 25285 was included for the quality control. The inoculum was prepared from a 48h anaerobic culture on enriched Brucella agar, adjusted in Brucella broth to the turbidity of 1 McFarland and swabbed onto Brucella agar supplemented with 5% sheep blood, vitamin K (0.001g/l) and hemin (0.005g/l). After displaying the Etest plastic strips onto the inoculated surface, the plates were incubated in jar, at 37^0C for 48-72h, in anaerobic atmosphere generated by *Genbox anaer* (BioMérieux, Marcy-l'Etoile, France). A resazurin strip was put into the jar as an indicator of anaerobiosis. The minimum inhibitory concentrations (MICs) were read after 48h of uninterrupted incubation (and in addition, after another 24h in case of metronidazole), at the point of complete inhibition of all bacterial growth. The interpretation of the MIC values followed the guidelines of the Clinical and Laboratory Standards Institute (CLSI), the former National Committee for Clinical Laboratory Standards (8).

Beta-lactamase test

The strains were tested for beta-lactamase production using the chromogenic cephalosporin method by streaking the colonies onto nitrocefin discs (AB Biodisk, Solna, Sweden) moistened with phosphate buffered saline, pH 7.3. *B. fragilis* ATCC 25285 was used as a positive control.

Statistical analysis

The Fisher exact probability test, with Yates' correction factor, was applied to evaluate the statistical significant relationship ($P<0.05$) between the antibiotic resistance (penicillin resistance, respectively) and the apartenence of the isolates to any particular species of *Prevotella*. The statistical software package used was Instat for MacIntosh, version 2.01.

Table 2. Distribution of *Prevotella* isolates by species and beta-lactamase production

Species	Number of isolates		
	Beta-lactamase negative	Beta-lactamase positive*	Total
P. melaninogenica	21	10	31
P. denticola	18	0	18
P. oralis	8	3	11
P. loscheii	5	0	5
Total	52	13	65

**The MICs range of benzylpenicillin in case of the beta-lactamase positive isolates of P. melaninogenica and P. oralis = 1-2μg/ml.*

Results

The MIC values of the tested antibiotics are presented in *Table 1*. According to the interpretative criteria of CLSI, 52 isolates were found to be sensitive to benzylpenicillin. Converting the MICs values into susceptibility categories, 2 isolates of *P. oralis* (including that one isolated with a *P. denticola* strain from the same abscess) and 3 isolates of *P. melaninogenica* were resistant to penicillin G, whereas 7 isolates of *P. melaninogenica* and another *P. oralis* strain were intermediate sensitive. These 13 strains were the only ones expressing beta-lactamase activity (*Table 2*). The results regarding the relationship between penicillin nonsusceptibility and apartenence to a certain species have indicated a significant association between penicillin resistance and *P. melaninogenica* isolates (P = 0.02), which was not found in *P. oralis* (P = 0.6). All strains were susceptible to cefoxitin, and clindamycin. Metronidazole was fully active on the *Prevotella* isolates and gave the same MIC values after 48h and 72h of incubation.

Discussion

This study assessed the activity of some commonly used antianaerobic drugs against *Prevotella* strains from abscesses of fascial spaces of the face and neck in Romanian patients. This series of 65 *Prevotella* isolates which belonged to the laboratory strains collection comprised no *P. intermedia*/*P. nigrescens* strains, since no isolate of these species had been recovered from the above mentioned cases of abscesses. Al-Nawas and Maeurer, evaluating the microbiological and clinical differences between severe life-threatening abscesses of the head and neck and submucous abscesses, noticed that the dominating anaerobic bacteria involved in the etiopathogeny of both these categories of abscesses were the *Prevotella* species, comprising nearly 30% of all isolated anaerobes (9). Other authors have also reported *Prevotella* as being the most prevalent microorganisms isolated from patients with odontogenic abscesses and cellulitis of the head and neck (6, 10).

Although the *in vitro* antibiotic susceptibility of the bacteria isolated from such infections is not routinely performed, it is recommended to investigate from time to time the local resistance patterns of aerobic and anaerobic isolates of clinical importance. The Etest is a convenient standardized method for testing the susceptibility of anaerobic microorganisms (11, 12) and clear endpoints were obtained for all antibiotics and strains tested in this study.

Several recent studies have shown that resistance to benzylpenicillin is not uncommon among *Prevotella* strains isolated from different infections, including the orofacial odontogenic infections (13, 14). In the present investigation, the overall rate of penicillin resistance was 20%, but some researchers have mentioned much higher values. Liu et al. found a penicillin resist-

ance rate of 62% among *Prevotella* isolates from patients hospitalized in a tertiary care medical centre in Taiwan in 2002, which actually increased to more than 90% in 2006 (7). Penicillin resistance of *Prevotella* isolates is mostly associated with beta-lactamase activity, which is almost the expression of the genes *cfxA* and *cfx A2* (15). In this research work, benzylpenicillin resistance has been detected only among the *P. melaninogenica* and *P. oralis* isolates and has been strongly correlated with the beta-lactamase production. However, several studies have indicated higher procents of beta-lactamase-positive *Prevotella* isolates. For example, a French research group found 58% of *Prevotella* isolates as being beta-lactamase producers (52% of the black-pigmented and 63% of the non-pigmented strains) (16), while another French team performing a multicentric survey on antimicrobial resistance of anaerobic Gram-negative bacilli isolated from hospitalized patients has reported 65% of the *Prevotella* isolates as being beta-lactamase-positive (17). Moreover, Bahar et al. detected this enzyme production in almost 70% of *P. melaninogenica* clinical isolates (18).

In the present research, all beta-lactamase positive strains were susceptible to cefoxitin and, in general, resistance to this antimicrobial agent has seldom been noticed. Gaetti-Jardim et al. evaluating the susceptibility of bacteria isolated from Brazilian subjects with endodontic infections found about 20% strains non-susceptible to cefoxitin (19). Liu et al. found 13% intermediate susceptible and 6% resistant *Prevotella* isolates associated with bloodstream infections at the National Taiwan University Hospital in 2006 (7).

In the last years, the investigators found different clindamycin resistant rates in *Prevotella* isolates from oral and extraoral infections, from less than 5% to more than 20% (10, 18, 20, 21). Clindamycin was fully active on these 65 *Prevotella* isolates and might be recommended in Romania as a suitable drug for the pyogenic head and neck infections (when the antimicrobial agents are needed in addition to the surgical treatment) produced by penicillin resistant bacteria or in patients allergic to beta-lactam antibiotics.

It is known that metronidazole is very active against anaerobes and the findings of this research were in agreement with this statement. Nevertheless, some recent studies have reported resistant anaerobic Gram-negative bacilli isolated from oral and non-oral infections (18, 22, 23). A research team investigating the presence of 5-nitroimidazole (*nim*) resistance gene by polymerase chain reaction demonstrated the inducible high level metronidazole resistance in a *Prevotella* strain isolated from a case of bacteremia (24). The same authors stressed on the importance of a prolonged incubation time (72h) for metronidazole susceptibility testing. In the present study, resistance to this antimicrobial agent was detected neither at 48h nor at 72h after incubation.

The results regarding the susceptibility of the 65 *Prevotella* isolates to cefoxitin, clindamycin and metronidazole were similar to those obtained recently by Bladino et al. when investigating the susceptibility of bacterial strains isolated from suppurative orofacial infections (25).

The data obtained in this study highly recommends the administration of antibiotics active against both aerobic and anaerobic bacteria, without forgetting the beta-lactamase producers, whenever the antimicrobial therapy in head and neck infections is required and is performed empirically. Future studies on larger samples are strongly needed in Romania to further explore the resistance of *Prevotella* and other anaerobic bacteria involved in severe oral and maxillofacial infections.

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Abbreviations

MIC - Minimum inhibitory concentration
CLSI - Clinical and Laboratory Standards Institute

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