

29

Original article

Antimicrobial susceptibility of anaerobic and capnophilic bacteria isolated from odontogenic abscesses and rapidly progressive periodontitis

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Abstract

In dentistry antimicrobials are used in the treatment of progressive periodontitis and odontogenic abscesses, therefore the susceptibility to commonly used antibiotics of capnophilic and anaerobic species causing these diseases should be investigated. The activity of penicillin, amoxycillin, cefoxitin, clindamycin, doxycycline, metronidazole and ciprofloxacin was investigated. One hundred and sixty four isolates from subgingival plaque samples of 66 patients with progressive periodontitis and 192 bacterial strains from pus of 74 patients with odontogenic abscesses were included in this study. The majority of species tested were gram-negative anaerobes (*Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp.), and were highly susceptible to clindamycin and metronidazole. Nearly 6% of the periodontal isolates and 22% of the bacteria obtained from pus samples produced β -lactamases. With the exception of the periodontopathogenic species *Actinobacillus actinomycetemcomitans* and *Eikenella corrodens*, clindamycin seemed to be a useful antibiotic and could be recommended for empirical antimicrobial treatment. © 1999 Elsevier Science B.V. and International Society of Chemotherapy. All rights reserved.

Keywords: Resistance patterns; Oral anaerobes; Progressive periodontitis; Odontogenic abscesses

1. Introduction

In dentistry antimicrobial chemotherapy is common as an adjunctive treatment of patients with odontogenic abscesses and progressive periodontal disease. In general these infections are caused by more than one species. Very often several different bacterial strains can be cultured, isolated and identified; most of them are anaerobes or capnophilic microorganisms.

Usually dentists choose from a limited spectrum of antibiotics such as penicillins, clindamycin, doxycycline and metronidazole. Additionally amoxycillin and ciprofloxacin have been recommended for the treatment

of periodontal disease [1,2]. Cefoxitin is an antimicrobial agent of importance in the treatment of infected maxillofacial surgery [3]. Over the last few years there have been some reports of oral species developing resistance to these antimicrobial agents [4–6]. Since it is known that resistance patterns vary with geographic districts [7], this study was performed to define the antibiotic susceptibility of anaerobic and capnophilic bacteria in Germany.

2. Material and methods

2.1. Patient specimens

Subgingival plaque samples of patients with progressive periodontal disease were obtained from the Depart-

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ment of Periodontology of Jena University. After removal of supragingival plaque, three paper points were inserted into a periodontal pocket for 20 s. The specimens were placed in 2 ml reduced transport fluid and incubated immediately.

The patients with odontogenic abscesses were recruited in the Department of Oral and Maxillofacial Surgery. Pus was aspirated after decontamination of the mucosa and transferred to Stuart's medium. The samples were plated onto solid culture media within 2 h.

2.2. Culture and identification of microorganisms

For the isolation and identification of the capnophilic and anaerobic bacteria the following culture media were used: Columbia agar with 8% sheep blood, chocolate agar, Schaedler agar with 8% sheep blood without antibiotics and with 7.5 mg/l vancomycin or 100 mg/l kanamycin. All plates were incubated with 5% CO₂ or anaerobically, as appropriate, for at least 5 days. Identification of the bacteria was based on colony morphology, cell morphology, respiratory requirements and biochemistry (ID 32 A, bioMerieux®, Marcy l'Etoile, France).

2.3. Antibiotic susceptibility test

Antibiotic susceptibilities were determined by the agar dilution technique. Wilkins–Chalgren agar plates with 10% sheep blood were supplemented with the appropriate antibiotic dilution to be tested. The inoculum was adjusted to 10⁷ bacteria/ml. Approx. 10⁴–10⁵ microorganisms were inoculated onto the plates by means of a multipoint inoculator. Plates were incubated in an anaerobic atmosphere for 48 h. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the antibiotic repressing visible growth. A Wilkins–Chalgren agar plate without antibiotic was used as a control plate. *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741 and *Clostridium perfringens* ATCC 13124 served as control strains. The following antimicrobial agents were studied: penicillin (Jenapharm, Jena, Germany), clindamycin (Upjohn, Heppenheim, Germany), doxycycline (Ratiopharm, Ulm, Germany) and metronidazole (Braun, Melsungen, Germany). The periodontal species were additionally tested against amoxicillin (Smith-Kline Beecham, Munich, Germany) and ciprofloxacin (Bayer, Leverkusen, Germany). The MIC of cefoxitin (MSD Sharp & Dohme GmbH, Haar, Germany) against the anaerobes from odontogenic abscesses was also determined. Based on the recommendations of

the NCCLS [8,9] the bacteria were considered to be resistant strains if MICs were: penicillin ≥ 2 mg/l, amoxicillin ≥ 16 mg/l, cefoxitin ≥ 64 mg/l, clindamycin ≥ 8 mg/l, doxycycline ≥ 8 mg/l, metronidazole ≥ 32 mg/l, and ciprofloxacin ≥ 4 mg/l.

The ability of the bacteria to synthesise a β-lactamase was assessed by means of nitrocefin disks (Becton Dickinson, Cockeysville, MD).

3. Results

Anaerobic and capnophilic species tested for antimicrobial susceptibilities were isolated from subgingival plaque samples of 66 patients with progressive periodontitis and from pus samples of 74 patients with odontogenic abscesses. 1–6 months prior to the collection of plaque samples, 21 of the periodontitis patients had been treated with metronidazole either locally or systemically. Nine patients with odontogenic abscesses had received tetracyclines and eight patients had undergone penicillin therapy.

The majority of the 164 species found in patients with periodontitis were black pigmented *Prevotella* species (*Prevotella intermedia*, *Prevotella nigrescens*) and *Porphyromonas gingivalis*. The number and MICs of these species and other isolates are shown in Table 1. In general the strains were sensitive to the range of antimicrobials. Only nine species from subgingival plaque samples, among them four *P. intermedia* and two *P. gingivalis* strains produced a β-lactamase. *Eikenella corrodens* and *Actinobacillus actinomycetemcomitans* were completely resistant to clindamycin and metronidazole, but the MICs of ciprofloxacin were very low. Four *P. gingivalis* strains, three black pigmented *Prevotella* spp., one *Actinomyces meyeri*, and one *Capnocytophaga* spp. showed resistance to doxycycline.

Beside the strains isolated from patients with periodontitis, 192 species from odontogenic abscesses were isolated and tested. The spectrum of microorganisms was different when compared to the subgingival samples. A large proportion of the species belonged to the non-pigmented *Prevotella* species such as *P. oralis*, *P. buccae*, and *P. denticola*. Only six *Porphyromonas* isolates (*P. gingivalis*, *P. asaccharolytica*, and *P. endodontalis*) could be detected. The group of 32 black pigmented *Prevotella* species consisted of 14 *P. melaninogenica*, 10 *P. intermedia/nigrescens* and seven *P. loeschei*. Gram-positive anaerobes included eight peptostreptococci and 21 rods (*Actinomyces* spp., *Eubacterium* spp., *Bifidobacterium* spp., *Propionibacterium* spp.). Table 2 shows the resistance patterns of the strains. With the exception of five gram-positive rods

Table 1

MIC (mg/l) of various antibiotics against anaerobic and capnophilic species isolated from subgingival plaque samples of patients with progressive periodontitis

	<i>n</i>	MIC range	MIC ₅₀	MIC ₉₀	Percentage resistant
Black pigmented					
<i>Prevotella</i> spp					
	52				
Penicillin		≤0.25–16	≤0.25	1	9.6
Amoxycillin		≤0.25–8	≤0.25	0.5	3.8
Clindamycin		≤0.125–8	≤0.125	≤0.125	1.9
Doxycycline		≤0.25–8	≤0.25	4	5.8
Metronidazole		≤0.25–2	≤0.25	0.5	0
Ciprofloxacin		0.25–16	0.5	8	19.2
<i>P. gingivalis</i>					
	26				
Penicillin		≤0.25–>64	≤0.25	≤0.25	7.7
Amoxycillin		≤0.25–16	≤0.25	≤0.25	7.6
Clindamycin		≤0.125–1	≤0.125	1	0
Doxycycline		≤0.25–32	≤0.25	16	15.4
Metronidazole		≤0.25–0.5	≤0.25	0.5	0
Ciprofloxacin		0.25–1	0.5	1	0
<i>Campylobacter rectus</i>					
	22				
Penicillin		≤0.25–16	≤0.25	2	18.2
Amoxycillin		≤0.25–8	≤0.25	1	4.5
Clindamycin		≤0.125–>16	≤0.125	>16	13.6
Doxycycline		≤0.25–4	≤0.25	2	0
Metronidazole		≤0.25–4	≤0.25	4	0
Ciprofloxacin		≤0.125–2	0.25	1	0
<i>Fusobacterium</i> spp					
	18				
Penicillin		0.25–4	≤0.25	≤0.25	5.6
Amoxycillin		≤0.25–2	≤0.25	≤0.25	0
Clindamycin		≤0.125–8	≤0.125	0.5	5.6
Doxycycline		≤0.25–1	≤0.25	0.5	0
Metronidazole		≤0.25–2	≤0.25	1	0
Ciprofloxacin		≤0.125–4	1	4	16.7
<i>A. actinomycetemcomitans</i>					
	16				
Penicillin		1–8	8	8	93.8
Amoxycillin		0.5–16	2	16	25.0
Clindamycin		1–>16	16	>16	87.5
Doxycycline		0.5–4	2	4	0
Metronidazole		8–32	16	>32	37.5
Ciprofloxacin		≤0.125–0.5	≤0.125	0.25	0
Gram-positive anaerobes					
	13				
Penicillin		≤0.25–1	≤0.25	1	0
Amoxycillin		≤0.25–0.5	≤0.25	≤0.25	0
Clindamycin		≤0.125–1	≤0.125	1	0
Doxycycline		≤0.25–8	0.5	2	7.7
Metronidazole		≤0.25–>32	8	>32	30.8
Ciprofloxacin		≤0.125–16	0.5	4	15.4
<i>E. corrodens</i>					
	9				
Penicillin		2–8	4	8	100
Amoxycillin		0.5–2	1	2	0
Clindamycin		>16	>16	>16	100
Doxycycline		1–2	2	2	0
Metronidazole		>32	>32	>32	100
Ciprofloxacin		≤0.125–0.5	≤0.125	0.25	0
<i>Capnocytophaga</i> spp					
	8				
Penicillin		≤0.25–4	≤0.25	4	12.5
Amoxycillin		≤0.25–2	≤0.25	2	0
Clindamycin		≤0.125–1	0.25	1	0
Doxycycline		≤0.25–8	≤0.25	8	12.5
Metronidazole		2–16	4	16	0
Ciprofloxacin		≤0.125–2	≤0.125	2	0

Table 2
MIC (mg/l) of various antibiotics against anaerobic and capnophilic species isolated from pus of patients with odontogenic abscesses

	n	Range of MIC	MIC ₅₀	MIC ₉₀	Percentage resistant
Non pigmented					
<i>Prevotella</i> spp					
Penicillin	41	≤0.25->64	0.5	32	36.6
Cefoxitin		≤0.5-16	2	8	0
Clindamycin		≤0.125-16	≤0.125	2	7.3
Doxycycline		≤0.25->32	0.5	>32	17.1
Metronidazole		≤0.25-4	1	2	0
Other gram-negative rods					
Penicillin	36	≤0.25->64	≤0.25	>64	41.7
Cefoxitin		≤0.5-64	1	32	5.6
Clindamycin		≤0.125-4	≤0.125	≤0.125	0
Doxycycline		≤0.25-32	0.5	16	25.0
Metronidazole		≤0.25->32	2	32	11.1
Black pigmented <i>Prevotella</i> spp					
Penicillin	32	≤0.25->64	≤0.25	16	34.4
Cefoxitin		≤0.5-32	1	8	0
Clindamycin		≤0.125-2	≤0.125	0.25	0
Doxycycline		≤0.25-16	0.5	4	3.1
Metronidazole		≤0.25-4	1	4	0
Gram-positive anaerobes					
<i>Fusobacterium</i> spp.					
Penicillin	29	≤0.25->64	≤0.25	8	41.4
Cefoxitin		≤0.5-64	2	16	6.9
Clindamycin		≤0.125->16	0.5	16	17.2
Doxycycline		≤0.25-16	1	16	31.0
Metronidazole		≤0.25->32	8	>32	37.9
<i>Veillonella</i> spp.					
Penicillin	28	≤0.25->64	0.5	>64	28.6
Cefoxitin		≤0.5->64	1	>64	10.7
Clindamycin		≤0.125-0.5	≤0.125	0.5	0
Doxycycline		≤0.25->32	2	>32	10.7
Metronidazole		≤0.25-8	4	8	0
<i>Fusobacterium</i> spp.					
Penicillin	13	≤0.25->64	≤0.25	>64	38.5
Cefoxitin		≤0.5->64	1	>64	30.8
Clindamycin		≤0.125-0.5	≤0.125	0.5	0
Doxycycline		≤0.25-32	≤0.25	4	7.7
Metronidazole		≤0.25-4	0.5	4	0
<i>Capnocytophaga</i> spp.					
Penicillin	7	≤0.25-32	≤0.25	32	57.1
Cefoxitin		≤0.5-8	2	8	0
Clindamycin		≤0.125-0.5	0.25	0.5	0
Doxycycline		≤0.25-8	0.5	8	14.3
Metronidazole		≤0.25->32	8	>32	42.9
<i>Porphyromonas</i> spp.					
Penicillin	6	≤0.25-4	0.5	4	16.7
Cefoxitin		≤0.5-4	≤0.5	4	0
Clindamycin		≤0.125-0.5	≤0.125	1	0
Doxycycline		≤0.25-1	≤0.25	1	0
Metronidazole		≤0.25-2	≤0.25	2	0

and three non pigmented *Prevotella* species, the MICs of clindamycin were very low. Among all test groups a total of 42 β-lactamase-positive strains were detected. The percentage was between seven (gram-positive anaerobes) and 31% (*Fusobacterium* spp.). Nearly a third of the *Fusobacterium* spp. and *Veillonella* spp. were resistant to cefoxitin. All *Prevotella* and *Porphyromonas* species tested were susceptible to metronida-

zole. The MIC of doxycycline was ≥ 8 mg/l for 19 gram-negative rods, three *Veillonella* spp. and nine gram-positive anaerobes.

These results did not show any effect of pretreatment with metronidazole. However, treatment of patients with tetracyclines or penicillin resulted in the isolation of more bacterial strains resistant to these antimicrobials than in patients without any prior antibiotic therapy.

4. Discussion

Most studies which have determined the susceptibility of anaerobic bacteria have been investigations of the bowel flora; a high percentage of organisms isolated and tested have been *Bacteroides* and *Clostridium* species [7,10]. In dentistry a different spectrum of anaerobic species is found and usually other antimicrobials are recommended for treatment. Since there are few studies on resistance patterns of capnophilic and anaerobic bacteria obtained from oral infections, the purpose of our study was to determine MICs of commonly used antibiotics against oral bacteria.

In general our results for the periodontal species agree with those of Miyake et al. [11]. But in contrast to ours these authors found lower clindamycin MICs against *A. actinomycetemcomitans* ($MIC_{50} = 4$) and they did not assess β -lactam antibiotics.

Compared with bacteria obtained from subgingival plaque samples, species isolated from odontogenic abscesses showed a higher resistance to the antimicrobials tested. Doxycycline and penicillins had been frequently used as antimicrobials in our Department of Oral and Maxillofacial Surgery. In 1988 a study performed by Gilmore et al. [12] found only 8.9% of the anaerobes tested had an $MIC \geq 1$ mg/l against penicillin. Today many species produce β -lactamases. Therefore penicillin and cefoxitin cannot be recommended for empirical antimicrobial therapy. The ability of species to synthesise β -lactamase should be determined before treatment with β -lactam antibiotics. If the infection is caused by gram-negative anaerobes, the use of metronidazole might be helpful. Our results agree with those of Roche et al. [13] who found anaerobes from odontogenic abscesses to be highly susceptible to metronidazole.

Clindamycin is a useful antibiotic in dentistry. It is able to penetrate bone and inhibit the formation of biofilms [14]. But *A. actinomycetemcomitans* and *E. corrodens*, both important species in cases of periodontitis, have a natural resistance to metronidazole and clindamycin. In cases of progressive periodontitis the presence of these two species should be excluded by culture or gene probe prior to treatment with clindamycin or metronidazole. If they are detected, Pavicic et al. [1] suggested using doxycycline or a combination of metronidazole and amoxicillin depending on sensitivities.

In contrast to the anaerobic species *A. actinomycetemcomitans* was susceptible to ciprofloxacin. Probably other newly developed quinolones might be very useful antimicrobials for the treatment of anaerobic infections. Spangler et al. [15] reported 489 anaerobes, which were high susceptible to trovafloxacin.

It must be realised that these in vitro-results cannot easily be extrapolated to the in vivo-situation. Bacteria can produce biofilms or are able to invade tissues and

so the penetration of antibiotics into cells and biofilms is important. In these sites the concentrations of many antibiotics are considerably lower than in the blood. Listgarten et al. [16] used 1 mg/l of tetracycline, metronidazole or penicillin as the breakpoint for resistance.

Even in odontogenic infections the determination of the microbial flora is necessary before antibiotic treatment. For calculated chemotherapy the anaerobes should be identified by culture or gene probe. If the antibiotic treatment fails, an aerobic, capnophilic and anaerobic culture should be carried out followed by determination of antimicrobial resistance. The antibiotic and route of administration should be chosen on the basis of the resistance data and the ability to penetrate to the site of the infection.

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