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Short communication

Antimicrobial susceptibility of 800 anaerobic isolates from patients with dentoalveolar infection to 13 oral antibiotics

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Introduction: The aim of this study was to determine the current antimicrobial susceptibility of the principle anaerobic pathogens involved in dentoalveolar infection, to 13 oral antibiotics, and to assess the value of each antibiotic in the management of the infection.
Methods: A total of 800 isolates from patients with dentoalveolar infection (*Prevotella* species, *Fusobacterium* species, *Porphyromonas* species and *Peptostreptococcus micros*) were tested for their susceptibility to amoxicillin, amoxicillin/clavulanate, cefaclor, cefuroxime, cefcapene, cefdinir, erythromycin, azithromycin, telithromycin, minocycline, levofloxacin, clindamycin, and metronidazole using an agar dilution method.
Results: Although the majority of *Fusobacterium* strains were resistant to erythromycin, azithromycin, and telithromycin, the remaining antibiotics demonstrated a high level of antimicrobial activity. *P. micros* and *Porphyromonas* species exhibited high susceptibility to all antibiotics tested in this study. In the case of *Prevotella* species, resistance to amoxicillin occurred in 34% of isolates and all of these resistant strains were found to produce β-lactamase. Susceptibility of *Prevotella* strains to cefaclor, cefuroxime, cefcapene, cefdinir, erythromycin, azithromycin, and minocycline was found to correlate with amoxicillin susceptibility. Amoxicillin/clavulanate, telithromycin, clindamycin, and metronidazole exhibited high antimicrobial activity even against amoxicillin-resistant strains of *Prevotella* species.
Conclusion: Amoxicillin would still be advocated therefore as being a suitable first-line agent, while reduced susceptibility of *Prevotella* strains remains a matter of concern with penicillins. Amoxicillin/clavulanate, clindamycin, and metronidazole are useful alternatives in combating the anaerobic bacteria involved in dentoalveolar infection.

Key words: *anaerobes; antimicrobial susceptibility; dental infection; penicillin*

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The majority of dentoalveolar infections are related to dental pulp necrosis and periodontal disease (2). These infections usually involve the bacteria residing in the oral cavity. While early microbiological investigations suggested that aerobic and oral streptococci were associated with dentoalveolar infection, recent improvements in sampling and culture techniques have shown that it is in fact strict anaerobes that predominate (1, 5, 7–11, 18). Although drainage is the most important factor in the treatment of dentoalveolar infections, systemic antibiotics may be prescribed to prevent the spread of infection and the onset of serious complications (5, 8, 17, 18). In circumstances where adequate drainage cannot be achieved, the role of antibiotic therapy is of even greater significance (8).

It is the antimicrobial susceptibility of the bacteria involved in these infections

that is the primary factor in determining the likely outcome of antibiotic treatment (6). Since antibiotic sensitivity results are generally not available for several days after the receipt of clinical samples, antibiotics are often prescribed empirically. Generally, members of the penicillin group are first-choice antibiotics because of their expected high antimicrobial activity, low incidence of adverse effects, and cost-effectiveness (5, 8, 9, 17, 18). Amoxicillin in particular, has been widely used in Japan and the UK, largely because of its better absorption from the gastrointestinal tract compared with other oral penicillin agents (8), although penicillin V has tended to be preferred in North America (17, 18). However, use of these antibiotics is avoided when patients are allergic or have previously exhibited adverse reaction following penicillin therapy (5, 17, 18). Furthermore, in situations where the causative agent is suspected of being penicillin-resistant, alternative antibiotics have to be considered. It is therefore somewhat surprising that there have been relatively few studies examining suitable alternatives to amoxicillin in managing dentoalveolar infections.

The purpose of this study was therefore to determine the current antimicrobial susceptibility of clinical isolates of *Fusobacterium* species, *Porphyromonas* species, *Prevotella* species, and *Peptostreptococcus micros* from patients with dentoalveolar infection, to 13 oral antibiotics. From the results, we assessed the value of each antibiotic in the management of dentoalveolar infections.

A total of 800 isolates (*Prevotella* species, 499; *Fusobacterium* species, 153; *P. micros*, 100; *Porphyromonas* species, 48) that were obtained from a total of 218 patients with dentoalveolar infection (endodontic origin 175, periodontic origin 33, periconotic origin 8, and post-operative wound infection origin 2) who were treated at Kanazawa University Hospital (Kanazawa, Japan), Noto General Hospital (Nanao, Japan), Kanazawa Social Insurance Hospital (Kanazawa), Komatsu Municipal Hospital (Komatsu, Japan), Shiraishi Dental Surgery (Kanazawa), and Shimada Dental Surgery (Komatsu) between January 1997 and December 2005 were examined in this study. Of the 800 strains, 626 were isolates from pus obtained by needle aspiration of the abscess, and the remaining 174 strains were isolated from the dental plaque of the patients. Bacteriological identification was performed using Rap ID ANA II (Remel, Lenexa, KS) (15).

Antimicrobial susceptibility of these isolates to amoxicillin (Astellas, Tokyo, Japan), amoxicillin/clavulanate (GlaxoSmithKline, Middlesex, UK), cefaclor (Shionogi, Osaka, Japan), cefuroxime (GlaxoSmithKline), cefcapene (Shionogi), cefdinir (Astellas), erythromycin (Shionogi), azithromycin (Pfizer, Tokyo, Japan), telithromycin (Astellas), minocycline (Wyeth, Tokyo, Japan), levofloxacin (Daiichi, Tokyo, Japan), clindamycin (Pfizer), and metronidazole (Shionogi) was determined using an agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI; formerly, NCCLS) (16). *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were used as quality control strains (16). Since the breakpoint for amoxicillin is not indicated in the CLSI documentation, this was determined based on a previous study (19) and determined to be: susceptible, ≤2 μg/ml or resistant, ≥4 μg/ml.

A nitrocefin disk test was performed to determine whether strains were positive for β-lactamase production as described previously (7). From recent microbiological studies, *Fusobacterium* species, *Porphyromonas* species, *Prevotella* species and *P. micros* are regarded as being the principle pathogens of dentoalveolar infection (1, 2) and recent investigations by our research group support this view (7–11).

Despite the fact that the majority of strains examined in this study were clinical isolates from pus, 174 strains not directly involved in the infection were obtained from the dental plaque of the associated tooth. Such plaque-isolated strains may well have similar properties to those strains involved in infection (2), although further study is required to investigate this. We have confirmed that there was no particular difference in antimicrobial susceptibility between the pus and plaque isolates tested in this study to the chosen antibiotics (data not shown).

In this study, all *Fusobacterium* isolates were identified as *Fusobacterium nucleatum*-like species. Some of these strains could have been *Fusobacterium necrophorum* although definitive discrimination between this species and *F. nucleatum* was not possible using the method described. Based on the breakpoints employed in our study, all but five strains of *Fusobacterium* species (n = 153) were susceptible to amoxicillin (Table 1). There were no *Fusobacterium* strains positive for β-lactamase production, and this is undoubtedly reflected by the high level of susceptibility to amoxicillin. Fusobacteria also exhibited good susceptibility to amoxicillin/clavulanate, a wide range of cephalosporin agents, minocycline, clindamycin, and metronidazole. However, the level of *Fusobacterium* susceptibility to cefaclor and cefuroxime was not so high. Erythromycin and azithromycin demonstrated reduced antimicrobial activity. Fusobacteria also had high minimum inhibitory concentrations (MICs) with telithromycin, and this may relate to its structural similarity to the erythromycin-like agents (3).

A total of 44 *Porphyromonas gingivalis* strains and four *Porphyromonas endodontalis* strains were examined; there was no apparent difference in the susceptibility pattern between these two species. Only one (2.1%) of the 48 *Porphyromonas* strains and none of the *Porphyromonas micros* strains was resistant to amoxicillin. As with *Fusobacterium*, β-lactamase production was not evident with any of these *Porphyromonas* strains. All of the other tested antibiotics were highly active against *Porphyromonas* species and *P. micros*, although cefaclor activity for *P. micros* was lower.

In contrast to the bacteria already mentioned, 168 (33.7%) of the 499 *Prevotella* strains were found to be resistant to amoxicillin. The resistant bacteria included 30 of 85 (35.3%) *Prevotella buccae*, seven of 36 (19.4%) *Prevotella denticola*, 42 of 132 (31.8%) *Prevotella intermedia/nigrescens*, 12 of 45 (26.7%) *Prevotella loescheii*, 33 of 88 (37.5%) *Prevotella melaninogenica*, 36 of 87 (41.4%) *Prevotella oralis/oris*, and eight of 26 (30.8%) unidentified *Prevotella* strains. β-lactamase production was evident in all of the 168 amoxicillin-resistant strains whereas β-lactamase production was only detected in 16 (4.8%) of the 331 amoxicillin-susceptible strains. It was, however, apparent that these 16 strains did exhibit relatively high MICs (1 or 2 μg/ml) for amoxicillin. These findings strongly suggest that production of β-lactamase is the principle mechanism of amoxicillin-resistance amongst these bacteria.

In the case of cephalosporin, all *Prevotella* species had low MIC_{50} and high MIC_{90} values, and this susceptibility profile was similar to that for amoxicillin. Moreover, all amoxicillin-resistant strains were resistant to the cephalosporins, while amoxicillin-susceptible strains were also cephalosporin-sensitive (Table 2). This would imply that resistance to cephalosporin may also be linked to β-lactamase production. The observation that all amoxicillin-resistant strains were similarly resistant to a wide range of cephalosporin

Table 1. Antimicrobial susceptibility of *Fusobacterium* spp., *Peptostreptococcus micros*, *Porphyromonas* spp., and various types of *Prevotella* to 13 oral antibiotics

Organisms (number of tested strains)	MIC_{50}/MIC_{90} (μg/ml)												
	AMPC	AMPC/CV	CCL	CXM	CFPN	CFDN	EM	AZM	TEL	MINO	LVFX	CLDM	MZ
Fusobacterium nucleatum/necrophorum (153)	0.06/1	0.06/0.5	2/8	1/8	0.25/1	0.25/2	16/>64	2/16	8/32	0.12/1	1/4	0.06/0.25	≤0.03/0.5
Peptostreptococcus micros (100)	0.12/0.5	0.06/0.25	2/8	0.12/1	0.06/0.5	0.12/0.5	1/2	1/2	≤0.03/0.06	≤0.03/0.5	0.5/1	0.12/0.25	0.12/1
Porphyromonas gingivalis/endodontalis (48)[1]	≤0.03/1	≤0.03/1	0.5/4	0.12/8	≤0.03/4	0.06/1	0.25/8	0.5/8	0.12/4	0.12/2	0.5/8	≤0.03/0.06	0.25/2
Prevotella buccae (85)	0.12/>64	0.25/4	2/>64	2/>64	1/64	1/64	4/64	2/16	0.5/4	0.12/16	1/8	≤0.03/0.25	0.5/2
P. denticola (36)	≤0.03/>64	0.06/4	1/>64	0.25/64	0.12/32	0.12/32	0.5/16	1/32	0.25/8	0.12/8	1/4	≤0.03/0.03	0.25/1
P. intermedia/nigrescens (132)	0.06/64	0.06/2	0.5/>64	0.25/>64	0.12/32	0.25/32	0.25/16	0.25/4	≤0.03/2	0.06/4	0.5/8	≤0.03/0.06	0.25/2
P. loescheii (45)	0.06/>64	0.06/4	1/>64	1/>64	0.06/16	0.12/32	2/64	2/16	0.25/4	0.12/8	2/8	≤0.03/0.06	0.25/1
P. melaninogenica (88)	0.12/64	0.12/2	1/>64	1/>64	0.25/32	0.12/32	2/32	1/16	0.25/4	0.12/8	1/8	≤0.03/0.12	0.25/2
P. oralis/oris (87)	0.25/>64	0.25/4	2/>64	2/>64	0.5/32	1/64	1/>64	1/>64	0.25/8	0.12/16	1/4	≤0.03/0.25	0.5/2
Unidentified *Prevotella* spp. (26)[2]	0.25/64	0.25/2	2/>64	0.5/>64	0.12/32	0.25/64	4/>64	2/>64	0.12/>64	0.12/16	2/16	≤0.03/>64	0.12/0.5

AMPC, amoxicillin; AMPC/CV, amoxicillin/clavulanate; CCL, cefaclor; CXM, cefuroxime; CFPN, cefcapene; CFDN, cefdinir; EM, erythromycin; AZM, azithromycin; TEL, telithromycin; MINO, minocycline; LVFX, levofloxacin; CLDM, clindamycin; MZ, metronidazole.
[1]*P. gingivalis*, 44 strains; *P. endodontalis*, four strains.
[2]Non-pigmented *Prevotella* that were not identified to species level.

Table 2. Antimicrobial susceptibility of amoxicillin-susceptible and amoxicillin-resistant *Prevotella* strains

Organisms (number of tested strains)	MIC_{50} / MIC_{90} μg/ml											
	AMPC/CV	CCL	CXM	CFPN	CFDN	EM	AZM	TEL	MINO	LVFX	CLDM	MZ
Pigmented *Prevotella*[1]												
Amoxicillin-susceptible strains (207)	≤0.03/0.25	0.25/2	0.12/2	≤0.03/1	0.06/0.5	0.5/16	0.5/8	0.12/2	0.06/2	1/8	≤0.03/≤0.03	0.25/2
Amoxicillin-resistant strains (94)	1/4	>64/>64	64/>64	16/64	16/>64	1/32	1/32	0.25/4	2/8	1/8	≤0.03/0.06	0.25/2
Non-pigmented *Prevotella*[2]												
Amoxicillin-susceptible strains (124)	0.12/0.25	1/8	1/8	0.12/1	0.25/1	2/64	2/16	0.5/4	0.06/4	1/4	≤0.03/0.12	0.5/2
Amoxicillin-resistant strains (74)	2/8	>64/>64	>64/>64	16/>64	32/>64	2/>64	2/>64	0.5/8	2/16	1/16	≤0.03/>64	0.5/2

[1]*Prevotella denticola*, *P. intermedia/nigrescens*, *P. loescheii*, and *P. melaninogenica*.
[2]*Prevotella buccae*, *P. oralis/oris*, and unidentified *Prevotella* spp.

agents is clinically important. In general, the newer generation cephalosporin agents have improved stability against some β-lactamases compared with the older generation agents (14). However, the results of this study would suggest that there is little value in the use of oral cephalosporins in managing dentoalveolar infection, particularly when penicillin-resistant strains are evident.

The majority of *Prevotella* strains were susceptible to amoxicillin/clavulanate, although amoxicillin-resistant strains did exhibit relatively higher MICs. As a result, amoxicillin/clavulanate would appear to be the most effective option in the treatment of dentoalveolar infection.

It has been reported that resistance against tetracycline and erythromycin may be genetically associated with the β-lactam-resistance mechanism in *Prevotella* species (4, 12). In the case of non-pigmented bacterial species, the present study demonstrated no difference in susceptibility against erythromycin and azithromycin for the amoxicillin-susceptible and resistant strains (Table 2). However, for the pigmented *Prevotella* species, the MICs of the amoxicillin-susceptible strains for erythromycin and azithromycin were notably higher than those of the amoxicillin-resistant strains. Consequently, the choice of these antibiotics in the management of dentoalveolar infections caused by amoxicillin-resistant strains would not be advocated based on our findings. Interestingly, both pigmented and non-pigmented *Prevotella* strains were susceptible to telithromycin, regardless of susceptibility to amoxicillin. From the viewpoint of antimicrobial activity, telithromycin may therefore be a more suitable option than use of the macrolide agents. In this study, the susceptibility to minocycline correlated well with amoxicillin, with reduced activity of minocycline for amoxicillin-resistant strains being evident. We previously recommended minocycline in the management of oral purulent infections (9). However, the results of this study do not support the use of tetracycline group agents, including minocycline, in cases where amoxicillin-resistant *Prevotella* strains are involved.

Levofloxacin has been widely prescribed by Japanese dental practitioners. In this study, however, the antimicrobial activity of levofloxacin was relatively low, suggesting a limited value of its use over other agents.

Prevotella strains were found to be highly susceptible to clindamycin and metronidazole, regardless of susceptibility to amoxicillin to which more than 10% of the unidentified strains exhibited resistance (Table 1). As mentioned previously, these antibiotics also had excellent activity against the other anaerobes, and would therefore serve as effective agents in treating dentoalveolar infection.

It is worth noting that various organisms are recovered from dentoalveolar infection (1, 10, 11), and some of the bacteria other than the anaerobic pathogens examined in this study could be resistant to the tested antibiotics. Moreover, *in vivo* activity of antibiotics can vary to that observed *in vitro* (13). These factors should be considered when choosing an antibiotic for the treatment of dentoalveolar infection, although the present results regarding *in vitro* susceptibility of the primary anaerobic pathogens can serve as a valuable aid in the selection of antibiotic therapy.

In conclusion, amoxicillin still exhibits a high level of activity against the majority of oral anaerobes, while reduced susceptibility of *Prevotella* strains could be a matter of concern with penicillins. Amoxicillin/clavulanate, clindamycin, and metronidazole would also be advocated as being useful alternatives for the management of dentoalveolar infection.

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