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Antimicrobial susceptibility of periodontopathogenic bacteria

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Objectives: **The aim of this study was to evaluate the resistance profiles of** Aggregatibacter (Actinobacillus) actinomycetemcomitans**,** Porphyromonas gingivalis **and** Prevotella intermedia/Prevotella nigrescens **and to detect possible changes in antibiotic resistance over the time period of 1991–2005.**

Methods: A. actinomycetemcomitans **(125 strains),** P. gingivalis **(152 strains) and** P. intermedia/P. nigrescens **(326 strains) isolated during the years 1991–2005 were tested for their susceptibility to amoxicillin/clavulanic acid, clindamycin, metronidazole, phenoxymethylpenicillin and tetracycline using the Etest.**

Results: **No antibiotic resistance was detected in** P. gingivalis**, whereas a few isolates of** P. intermedia **were not susceptible to clindamycin (0.9%), phenoxymethylpenicillin (13.5%) or tetracycline (12.6%). Amoxicillin/clavulanic acid, tetracycline and metronidazole were the most effective antibiotics against** A. actinomycetemcomitans **with 0%, 0.8% and 20.8% non-susceptible isolates, respectively. However, 88% of the** A. actinomycetemcomitans **isolates were non-susceptible to phenoxymethylpenicillin and 88% to clindamycin. When strains isolated in the years 1991–94 were compared with those isolated in the years 2001–04, there was no statistically significant difference in the percentage of** A. actinomycetemcomitans **strains non-susceptible to clindamycin, metronidazole or phenoxymethylpenicillin, or in the percentage of** P. intermedia **strains non-susceptible to phenoxymethylpenicillin or tetracycline (**$P > 0.4$ **each).**

Conclusions: **Increasing antibiotic resistances in periodontopathogenic bacteria are not yet a problem in the Northern part of Switzerland.**

Keywords: Etest, MIC, *Aggregatibacter (Actinobacillus) actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*

Introduction

The oral cavity is colonized by a diverse microflora. Several bacterial species have been implicated as causative agents of various oral diseases. Porphyromonas gingivalis and Prevotella intermedia/Prevotella nigrescens are black-pigmented, strictly anaerobic Gram-negative rods. P. gingivalis is frequently associated with aggressive periodontitis, whereas P. intermedia has also been implicated as a causative agent of periodontitis.[1] These species are also among the predominant bacteria isolated from odontogenic abscesses.[2] Aggregatibacter (Actinobacillus) actinomycetemcomitans is a Gram-negative, capnophilic coccobacillus mainly associated with localized aggressive periodontitis.[3] However, it has also been isolated from other oral sites than the periodontal pocket,[4] as well as from non-oral infections such as abscesses and endocarditis.[5]

In the therapy of orofacial odontogenic abscesses, which are mostly polymicrobial infections, antibiotics are often given in addition to surgical drainage.[6] In the case of periodontal diseases, most patients respond well to a therapy consisting of mechanical and surgical debridement. However, antimicrobial agents are used as adjuncts after conventional treatment failures and in aggressive periodontitis.[7]

As for numerous other microorganisms, increasing antibiotic resistance among oral bacteria may also be a potential major public health problem; however, only one study comparing resistances in oral bacteria has been published so far, comparing isolates collected from 1980–85 to isolates from 1991–95.[8]

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Additionally, the prevalence of resistance varies between geographic locations.[9] Therefore, the aim of this study was to evaluate the resistance profile of oral isolates of A. actinomycetemcomitans, P. gingivalis and P. intermedia from the North-western part of Switzerland and to detect possible changes in antibiotic resistance over the time period of 1991–2005.

Materials and methods

Isolation of bacteria

One hundred and twenty-five strains of A. actinomycetemcomitans, 152 strains of P. gingivalis and 326 strains of P. intermedia/P. nigrescens have been isolated from various intraoral sites (Table 1) during the years 1991–2005 and stored at −70°C (Microbank, Chemie Brunschwig, Basel, Switzerland).

P. gingivalis and P. intermedia/P. nigrescens were isolated on human blood agar plates [Columbia Agar Base (BBL Becton–Dickinson, Allschwil, Switzerland) supplemented with 5 mg/L haemin, 1 mg/L menadione and 50 mL/L human blood]. For the isolation of A. actinomycetemcomitans, trypticase-soy-bacitracin-vancomycin-agar plates were used.[10] Identification of the pigmented colonies and of A. actinomycetemcomitans was based on Gram's stain and cell morphology, aerotolerance, production of catalase and on biochemical reactions (rapid ID 32 A; bioMérieux, Meyrin, Switzerland) and/or with a selected set of biochemical reactions (Rosco, Mecolab, Hölstein, Switzerland). The black-pigmented Prevotella-species P. intermedia and P. nigrescens were not differentiated and are referred to as P. intermedia.

One isolate per patient was selected for the antimicrobial susceptibility testing.

Antimicrobial susceptibility testing

The susceptibilities of the selected bacterial species were determined by the Etest method. The antibiotics used were: amoxicillin/clavulanic acid (2/1), clindamycin, metronidazole, phenoxymethylpenicillin and tetracycline (DMD, Arlesheim, Switzerland), the most commonly used antibiotics in dentistry in Switzerland.[11] Inocula of the test strains were prepared in 0.9% NaCl, adjusted to a turbidity equivalent to that of at least a 0.5 McFarland standard and inoculated on Brucella plates supplemented with 50 mL/L human blood and 1 mg/L menadione. One Etest strip of the respective antibiotic was placed in the middle of each plate and the plates were then incubated under appropriate conditions (air þ 10% CO_2 for A. actinomycetemcomitans and 10% CO_2, 10% H_2, 80% N_2 for anaerobes and for A. actinomycetemcomitans in the case of testing metronidazole, respectively) for at least 48 h. The MICs were determined according to the manufacturer's instructions.

Bacteroides fragilis ATCC 25285 was included in each run as quality control.

The breakpoints for susceptibility of anaerobes to the antibiotics were applied for P. gingivalis and P. intermedia as recommended by the Clinical and Laboratory Standards Institute.[12] In the case of A. actinomycetemcomitans, the interpretive criteria for the HACEK group (i.e. the aphrophilus cluster of the genus Haemophilus, A. actinomycetemcomitans, Cardiobacterium species, Eikenella corrodens and Klingella species) were applied for amoxicillin/clavulanic acid and penicillin, whereas for metronidazole those for anaerobes were used. As no interpretive criteria exist for clindamycin and tetracycline, the interpretive criteria for anaerobes were applied.[12,13] Concentrations of antimicrobial agents achievable in the gingival crevicular fluid mostly correlate well with the interpretive categories by the Clinical and Laboratory Standards Institute.[14]

Data analysis

The numbers of susceptible and non-susceptible isolates during the years 1991–94 and 2001–04 were compared by the two-tailed Fisher's exact test using the software Analyse-it for Microsoft Excel (Analyse-it for Microsoft Excel, Leeds, UK, Version 1.72).

Results

Table 2 shows the in vitro susceptibility of the three oral pathogens A. actinomycetemcomitans (n ¼ 125), P. gingivalis (n ¼ 152) and P. intermedia (n ¼ 326) to the five antibiotics tested. All antibiotics were highly active against P. gingivalis. Amoxicillin/clavulanic acid, clindamycin and metronidazole were very effective against P. intermedia, while ≤13% of the isolates were not susceptible to either phenoxymethylpenicillin or tetracycline. A. actinomycetemcomitans showed a high level of resistance to phenoxymethylpenicillin and clindamycin. All isolates were susceptible to amoxicillin/clavulanic acid and ≥80% to metronidazole. One A. actinomycetemcomitans isolate had an intermediate resistance to tetracycline, whereas all other isolates were susceptible to this antibiotic.

The numbers of susceptible and non-susceptible (resistant plus intermediate) isolates during the years 1991–94 versus 2001–04 were compared in order to detect a potential increase in antibiotic resistance over time. No statistically significant difference was detected in the susceptibility of A. actinomycetemcomitans or P. intermedia (Table 3).

Discussion

The black-pigmented anaerobe P. gingivalis was highly susceptible to the tested antibiotics amoxicillin/clavulanic acid,

Table 1. Oral sources of the bacterial isolates tested

	Periodontal diseases	Abscesses	Other oral diseases	Diagnosis not specified[a]
A. actinomycetemcomitans	118	2	1	4
P. gingivalis	128	4	5	15
P. intermedia/P. nigrescens	256	19	27	24
Total	502	25	33	43

[a]For some isolates, especially for strains isolated in the early 90s, it was not possible to retrospectively obtain the exact clinical diagnosis.

Table 2. In vitro susceptibility of the oral bacterial isolates

Microorganism and antibiotic	MIC (mg/L)[a] range	50%	90%	Percentage susceptible
A. actinomycetemcomitans				
amoxicillin/clavulanic acid	, 0.016 to 3	1	2	100
clindamycin	, 0.016 to . 256	12	32	12[b]
metronidazole	, 0.016 to . 256	2	128	79.2
phenoxymethylpenicillin	, 0.016 to 64	4	16	12
tetracycline	, 0.016 to 12	0.38	0.75	99.2[b]
P. gingivalis				
amoxicillin/clavulanic acid	, 0.016 to 0.064	, 0.016	, 0.016	100
clindamycin	, 0.016 to 0.125	, 0.016	, 0.016	100
metronidazole	, 0.016 to 0.016	, 0.016	, 0.016	100
phenoxymethylpenicillin	, 0.016 to 0.047	, 0.016	, 0.016	100
tetracycline	, 0.016 to 2	0.023	0.19	100
P. intermedia/P. nigrescens				
amoxicillin/clavulanic acid	, 0.016 to 0.75	0.016	0.047	100
clindamycin	, 0.016 to . 256	, 0.016	, 0.016	99.1
metronidazole	, 0.016 to 0.5	0.047	0.125	100
phenoxymethylpenicillin	, 0.016 to 64	0.016	2	86.5
tetracycline	, 0.016 to 32	0.064	6	87.4

[a]50% and 90% indicate the MIC values at which 50% and 90% of isolates were inhibited, respectively.
[b]No available interpretive criteria. The interpretive criteria for anaerobic bacteria were applied.

Table 3. Total number of isolates during the years 1991–94 and 2001–04, the number of non-susceptible isolates and the corresponding P value

Microorganism and antibiotic	Number of isolates 1991–94 total	non-susceptible	Number of isolates 2001–04 Total	non-susceptible	P
A. actinomycetemcomitans	77		21		
clindamycin		67		19	1
metronidazole		17		3	0.65
phenoxymethylpenicillin		66		20	0.44
P. intermedia/P. nigrescens	143		116		
phenoxymethylpenicillin		20		17	1
tetracycline		18		15	1

clindamycin, metronidazole, phenoxymethylpenicillin and tetracycline. This is in accordance with other studies which show that this bacterium is highly susceptible to various antibiotics.[2,8,15–18] Other investigators, however, detected resistances in subgingival P. gingivalis isolates and isolates from odontogenic abscesses.[9,19] In this context, it is important to note that P. gingivalis has recently been shown to be capable of conjugal transfer of chromosomal and plasmid DNA which would provide an effective way to also transfer resistance determinants.[20]

The other black-pigmented anaerobe, P. intermedia, was also very susceptible to the antibiotics amoxicillin/clavulanic acid, clindamycin and metronidazole, whereas □ 13% of the isolates were non-susceptible to phenoxymethylpenicillin or tetracycline. Amoxicillin/clavulanic acid, metronidazole and clindamycin are generally regarded as highly effective against Prevotella species, including P. intermedia, with the majority of oral origin susceptible to these antibiotics.[9,15–17,21–23] Tetracycline was less active in this study. Tetracyclines are frequently used in the treatment of periodontal diseases, also in the form of local delivery devices.[24] The proportion of P. intermedia isolates resistant to tetracycline and its derivatives, primarily doxycycline and minocycline, differed in other studies,[9,17,21,22] which was mainly attributed to the varying degree of their use in different countries.[9] Prevotella species are known to produce β-lactamases.[2,25,26] This may in part explain the resistance of the

P. intermedia isolates in this study to penicillin, as all isolates were susceptible to amoxicillin/clavulanic acid. With 13% non-susceptible isolates, we detected a relatively low resistance to penicillin compared with findings from other parts of the world.[9,17,21–23]

A. actinomycetemcomitans was the least susceptible species, with amoxicillin/clavulanic acid and tetracycline being the most effective antibiotics. The high susceptibility of A. actinomycetemcomitans to these two antibiotics is corroborated by other studies.[9,17,27] One notable exception, however, was that 100% of subgingival A. actinomycetemcomitans isolated from Spanish patients grew on tetracycline-containing agar plates versus 0% isolated from Dutch patients.[9] Approximately 20% of the A. actinomycetemcomitans isolates from Basel were not susceptible to metronidazole. Data on the susceptibility of A. actinomycetemcomitans to this antibiotic vary.[9,17,19,27,28] A. actinomycetemcomitans was incubated differently in these studies, either anaerobically, as in our study, or under capnophilic/microaerophilic conditions. Metronidazole, however, is a prodrug that has to be activated by a redox-reaction. This reduction takes place most effectively under anaerobic conditions.[29] Although the metabolism of A. actinomycetemcomitans under in vivo conditions is not known, metronidazole plus amoxicillin show clinical success in patients with periodontal diseases and are therefore recommended to eradicate this bacterium.[30] The least effective antibiotics were phenoxymethylpenicillin and clindamycin. Although penicillins are commonly used against microorganisms of the HACEK group,[13] only 12% of the oral isolates were susceptible to this antibiotic. This is in line with the study of Eick et al.[19] where only 6.2% of the A. actinomycetemcomitans isolates were susceptible to penicillin. Higher susceptibility rates were reported by Madinier et al.[27] with 60% and by van Winkelhoff et al.[9] with 57.1% of the Dutch and 100% of the Spanish isolates to be susceptible to penicillin. There are also diverging results concerning the effectiveness of clindamycin against A. actinomycetemcomitans.[9,19,31] van Winkelhoff et al.[9] found no A. actinomycetemcomitans isolates from The Netherlands or from Spain growing on clindamycin-containing agar plates. On the other hand, Miyake et al.[31] and Eick et al.[19] found this bacterium to be very resistant against clindamycin.

At many sites in the oral cavity, oral bacteria are organized as a complex biofilm. Models of oral multispecies biofilms exist and the effects of antimicrobial substances were studied.[24,32,33] Most, but not all, species of subgingival bacteria were considerably more resistant in biofilms than in planktonic cultures.[24,33] MIC values presented in this study may also differ for A. actinomycetemcomitans, P. intermedia and P. gingivalis strains growing in biofilms. However, bacteria in the subgingival plaque can grow in different forms, either on the tooth surface as a biofilm or more loosely organized in the non-adherent plaque and antibiotic resistances may differ between these two locations. Furthermore, A. actinomycetemcomitans and P. gingivalis are able to adhere and invade oral epithelial cells and can even grow intracellularly.[34] The efficacy of antibiotics with well known intracellular activities was tested against these bacteria within epithelial cells. It could be shown that the elimination of intracellular bacteria by systemic antibiotics alone is problematic.[35] All these factors illustrate why the clinical success in the management of periodontal diseases remains dependent on mechanical treatment.[7]

The emergence of resistant pathogens is not only of concern in medicine, but also in dentistry as it may be one reason for treatment failure. Oral bacteria could also play an important part in the spread of resistance genes. Viridans streptococci, which are part of the normal flora, may act as a reservoir of antibiotic resistance genes which subsequently may be transferred to pathogens such as Streptococcus pneumoniae and Streptococcus pyogenes.[36] Genetic exchange between Treponema denticola and Streptococcus gordonii has been demonstrated in biofilms in vitro.[37] Streptococci and other oral bacteria may transfer resistance genes to intestinal bacteria.[38]

No increase in the prevalence of non-susceptible strains could be detected in this study when P. intermedia or A. actinomycetemcomitans strains isolated during the years 1991–94 were compared with those isolated during 2001–04. This is in contrast to Walker,[8] the only study so far in which data of resistances in oral bacteria were compared over time. Walker compared □ 900 periodontal strains collected from 1980–85 to □ 300 periodontal strains from 1991–95, and reported significant increases ($P < 0.05$) in the percentage of strains resistant to tetracycline, doxycycline and amoxicillin. Furthermore, he could detect a trend towards an increase in resistance to penicillin, but there was no significant change in resistance to either erythromycin or clindamycin. In the same study, Walker[8] compared several earlier studies and concluded that antibiotic resistances to tetracyclines and penicillins have increased in the periodontal flora.

Results of the two studies are difficult to compare as Walker[8] analysed various oral bacterial species; we concentrated on the three oral pathogens A. actinomycetemcomitans, P. intermedia and P. gingivalis only. The two black-pigmented bacteria were also tested by Walker in 1996. P. gingivalis was susceptible to all seven antibiotics tested, including tetracycline, penicillin-G and clindamycin, while the percentage of P. intermedia isolates susceptible to these three antibiotics was in the same range as in this study. A. actinomycetemcomitans was not analysed.[8] In addition to different methods and breakpoints, it is conceivable that there can be an increase in antibiotic resistance for the total oral flora, but not for individual species such as P. gingivalis, P. intermedia and A. actinomycetemcomitans. It should be noted that more than 500 bacterial species or phylotypes inhabit the oral cavity.[39] Therefore, to fully evaluate an increase in antibiotic resistance, commensals but also other periodontopathogenic species such as Tannerella forsythia, Fusobacterium nucleatum, Campylobacter rectus and T. denticola need to be tested as well.

The level of resistance varies between countries, which can be attributed to the different use of antibiotics.[9] Among European countries, Switzerland is the country with the lowest antibiotic consumption per capita,[40] which may in part explain why antibiotic resistance did not increase among isolates from the Basel area. Nevertheless, given the increase in antibiotic resistances seen for other pathogens, a prudent use of antibiotics in the management of periodontal diseases is still advisable.

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Transparency declarations

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