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Heterogeneity of *Actinobacillus actinomycetemcomitans* Strains in Various Human Infections and Relationships between Serotype, Genotype, and Antimicrobial Susceptibility

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***Actinobacillus actinomycetemcomitans*, an oral pathogen, only occasionally causes nonoral infections. In this study 52 *A. actinomycetemcomitans* strains from 51 subjects with nonoral infections were serotyped and genotyped by arbitrarily primed PCR (AP-PCR) to determine whether a certain clone(s) is specifically associated with nonoral infections or particular in vitro antimicrobial susceptibility patterns. The promoter structure of leukotoxin genes was additionally investigated to find the deletion characteristic of highly leukotoxic *A. actinomycetemcomitans* strains. The nonoral *A. actinomycetemcomitans* strains included all five known serotypes and nonserotypeable strains, the most common serotypes being b (40%) and c (31%). AP-PCR distinguished 10 different genotypes. *A. actinomycetemcomitans* serotype b strains were more frequently found in blood samples of patients with bacteremia or endocarditis than in patients with focal infections. One AP-PCR genotype was significantly more frequently found among strains originating in focal infections than in blood samples. Resistance to benzylpenicillin was significantly more frequent among *A. actinomycetemcomitans* serotype b strains than among strains of other serotypes. No differences in the leukotoxin gene promoter region or benzylpenicillin resistance between nonoral and oral *A. actinomycetemcomitans* strains were observed. Nonoral *A. actinomycetemcomitans* strains showed great similarity to the oral strains, confirming that the oral cavity is the likely source of nonoral *A. actinomycetemcomitans* infections. The predominance of serotype b strains in endocarditis and bacteremia supports the hypothesis of a relationship between certain *A. actinomycetemcomitans* clones and some nonoral infections. The mechanisms behind the exceptionally high rate of occurrence of benzylpenicillin resistance among *A. actinomycetemcomitans* serotype b strains are to be elucidated in further studies.**

Actinobacillus actinomycetemcomitans, a gram-negative facultatively anaerobic coccobacillus, is an important pathogen in periodontitis, a chronic tissue-destructive infection which may eventually lead to the loss of teeth (16, 44). Despite the rather common presence of the organism in the oral cavity, a literature review for nonoral *A. actinomycetemcomitans* infections revealed that less than 200 cases were reported during the last 30 years. These infections include endocarditis (9, 22, 23, 30, 40), pericarditis (20), pneumonia (43), septicemia (22, 39), and abscesses in various body sites (22). Approximately 0.6% of infective endocarditis cases are caused by *A. actinomycetemcomitans* (12). The rare recovery of *A. actinomycetemcomitans* from nonoral infections may be due to difficulties in growing and identifying the organism, and therefore, it may remain unrecognized or is misidentified (12, 25). It is also possible that only certain *A. actinomycetemcomitans* clones possess the capacity to cause invasive infections.

Of the five currently known *A. actinomycetemcomitans* serotypes (serotypes a through e) (32, 44), the most prevalent ones in the oral cavity are serotypes a, b, and c, making up more than 80% of strains at almost equal frequencies (32, 33). Serotype b is associated with periodontitis, and serotype c seems to be particularly frequent in periodontally healthy subjects (1, 44). The only study on the distribution of the three most common serotypes of *A. actinomycetemcomitans* in nonoral infections revealed a predominance of serotype c (45). However, no information on the presence of the two novel *A. actinomycetemcomitans* serotypes, serotypes d and e (15, 32), in nonoral infections is available.

Some oral *A. actinomycetemcomitans* clones may exert an elevated pathogenic potential to cause periodontal destruction, as suggested by several recent studies in which particular genotypes of the organism were associated with certain forms of periodontal diseases or gingival health (4, 8, 13, 19). One of the major virulence determinants of *A. actinomycetemcomitans* is leukotoxin, which is specifically cytotoxic to human polymorphonuclear leukocytes and monocytes (6, 37). All *A. actinomycetemcomitans* strains seem to have genes that code for leukotoxin (31). However, a deletion in the leukotoxin gene promoter region leads to expression of increased leukotoxic activity (7). Recently, colonization with *A. actinomycetemcomitans* strains with the particular deletion was reported to predict conversion from health to periodontal destruction in children (8).

The oral cavity is the ecological niche for *A. actinomycetemcomitans*. Therefore, it is likely that the source of nonoral *A. actinomycetemcomitans* infections is the oral cavity, especially in patients with periodontitis. A statement of the American Heart Association concerning prevention of infective endocarditis by prophylactic administration of amoxicillin (11) is complied with in dental care. There is a consensus among studies from different geographical locations that amoxicillin, among other ampicillin-group penicillins, generally exhibits good in vitro activity against oral strains of *A. actinomycetemcomitans*,

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TABLE 1. Origins of 52 nonoral *A. actinomycetemcomitans* strains from 51 subjects

Infection	Sample source	No. of subjects with depicted infection
Endocarditis	Blood	13
Pneumonia or lung abscess	Aspirated pus, tissue biopsy specimen	6
Thoracic wall infection	Aspirated pus, tissue biopsy specimen	3
Elbow or finger abscesses	Aspirated pus	2
Infection of neck	Tissue biopsy specimen	3
Osteitis[a]	Excised bone	2[b]
Bacteremia of unknown origin	Blood	22
Total		51

[a] Osteitis of the hand and an unknown site.
[b] One of the two patients was infected with two strains of different serotypes, serotypes b and c.

although some amoxicillin-resistant *A. actinomycetemcomitans* strains have been detected (29, 34, 41).

The aim of the present study was to characterize serotypically and genotypically *A. actinomycetemcomitans* strains isolated from nonoral infections to find out if a certain serotype(s) or genotype(s) is specifically associated with nonoral *A. actinomycetemcomitans* infections. We also analyzed the promoter structure of *A. actinomycetemcomitans* leukotoxin genes in order to find signs of elevated pathogenicity among nonoral strains in comparison with the pathogenicities of oral strains. Additionally, to facilitate prediction of optimal candidates for antimicrobial therapy in these infections, we compared the antimicrobial susceptibilities of *A. actinomycetemcomitans* strains in relation to their recovery from nonoral or oral infections and between serotypes and genotypes.

MATERIALS AND METHODS

***A. actinomycetemcomitans* strains.** The present collection of bacteria comprised 52 *A. actinomycetemcomitans* strains from 51 subjects diagnosed with various nonoral infections (Table 1) and 21 oral *A. actinomycetemcomitans* strains from 21 subjects. *A. actinomycetemcomitans* JP2, which expresses the deletion in the leukotoxin gene promoter region characteristic of the highly leukotoxic *A. actinomycetemcomitans* strains (7), was used as a reference strain in the PCR analysis of the leukotoxin gene promoter structure.

The *A. actinomycetemcomitans* strains from nonoral infections were obtained from geographically distant locations: the Culture Collection at the University of Göteborg in Göteborg, Sweden (Sweden, $n = 24$; Austria, $n = 1$; Germany, $n = 2$; and United States, $n = 1$), from international research groups that possess published or unpublished data on nonoral *A. actinomycetemcomitans* infections (Iceland, $n = 4$; Belgium, $n = 2$; New Zealand, $n = 6$; and Taiwan, $n = 4$), and from patients with unpublished *A. actinomycetemcomitans* infections from hospitals in Finland ($n = 8$). *A. actinomycetemcomitans* strains were recovered from both mono- and polyinfections, although the possible existence of copathogens was not always known. After arrival at our laboratory the cultures of nonoral *A. actinomycetemcomitans* strains were plated on Trypticase soy-serum-bacitracin-vancomycin (TSBV) agar plates (35) and the plates were incubated in 5% CO_2 in air at 37°C for 2 to 3 days. The species identification was confirmed as described previously (32). Briefly, colonies on TSBV agar plates were identified as *A. actinomycetemcomitans* if they presented a typical colony morphology, a positive catalase reaction, and a negative result for lactose fermentation. Subcultures starting from a single colony per sample were preserved in 20% skim milk at −70°C until they were used.

A total of 21 oral *A. actinomycetemcomitans* strains comprised the reference material in the analysis of the leukotoxin gene promoter structure and for antimicrobial susceptibility testing and were selected from our strain collection to comply with the serotype and genotype distributions of the *A. actinomycetemcomitans* strains from nonoral infections. The oral strains originated in subjects with periodontitis ($n = 19$), gingivitis ($n = 1$), or a healthy periodontium ($n = 1$) (age range, 14 to 71 years) and had been identified as described earlier (32).

Serotyping. Serotyping of *A. actinomycetemcomitans* strains was performed by using autoclaved whole *A. actinomycetemcomitans* cell antigen extract and serotype-specific rabbit antisera in an immunodiffusion assay as described previously (32).

AP-PCR genotyping. The random sequence oligonucleotide OPA-13 (5′-CA GCACCCAC-3′) (Operon Technologies, Inc., Alameda, Calif.) was used as a primer in the arbitrarily primed PCR (AP-PCR) analysis as previously reported in detail (2, 28).

Analysis of leukotoxin promoter structure by PCR amplification. The primer pair 5′-ATA TTA AAT CTC CTT GT-3′ and 5′-ACC TGA TAA CAG TAT T-3′ (7) was used to amplify a DNA fragment from the leukotoxin promoter region of *A. actinomycetemcomitans* strains as described earlier (4).

Antimicrobial susceptibility testing. The MICs of six antimicrobial agents for the 52 nonoral *A. actinomycetemcomitans* strains and 21 oral *A. actinomycetemcomitans* strains were determined by the agar dilution susceptibility testing method approved by the National Committee for Clinical Laboratory Standards (NCCLS) (26) with *Haemophilus* test medium. *Haemophilus influenzae* ATCC 49247 and ATCC 49766 and *Haemophilus aphrophilus* ATCC 13252 and NCTC 5906 were included as controls. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included as additional control strains. The six antimicrobial agents, supplied as standard powders by several manufacturers, included benzylpenicillin, amoxicillin, tetracycline, metronidazole, azithromycin, and trovafloxacin. The antimicrobial agent concentrations in the agar medium ranged from 0.06 to 32.0 mg/liter for all other agents except for metronidazole, which was used at concentrations ranging from 0.25 to 128.0 mg/liter. *A. actinomycetemcomitans* strains were grown on brucella blood agar plates in 5% CO_2 in air at 37°C for 48 h. The bacterial masses from the plates were harvested, the masses were adjusted into suspensions with turbidities equal to the turbidity of a McFarland 0.5 standard, and the final inoculum (10^4 CFU per spot) was delivered onto the agar plates with a multipoint inoculator. After incubation in 37°C in 5% CO_2 in air (metronidazole-containing plates, however, were incubated in anaerobic jars filled with mixed gas [85% N_2, 10% H_2, 5% CO_2]) for 48 h, the MIC results were interpreted according to NCCLS guidelines (26, 27).

Statistical methods. The statistical significance of the differences between the frequency distributions were determined by chi-square statistics and Fisher's exact test.

RESULTS

Strains of all five known serotypes and, additionally, a few nonserotypeable strains were recovered from among the 52 nonoral *A. actinomycetemcomitans* strains that originated from 51 subjects. Serotypes b (21 of 51; 41%) and c (16 of 51; 31%) were the most frequent serotypes in the subjects, whereas strains of serotypes a (8 of 51; 16%), d (4 of 51; 8%), and e (1 of 51; 2%) and nonserotypeable strains (2 of 51; 4%) occurred less commonly. One subject harbored two serotypes, serotypes b and c. Oligonucleotide OPA-13 distinguished a total of 10 AP-PCR genotypes (Fig. 1), with 1 to 3 genotypes within each serotype, among the 52 strains.

To determine possible differences in the serotype distributions in relation to the recovery site, the *A. actinomycetemcomitans* strains were divided into two groups according to their detection either from blood or from focal infections (Table 1). Table 2 shows the serotype and genotype distributions of the strains in blood and in focal infections when one *A. actinomycetemcomitans* strain per subject was examined; only strains from the subject colonized with strains of two different serotypes were excluded. Serotype b was (phi-square = 0.0714; $P = 0.0553$) more frequent in blood samples (17 of 35; 49%) than in focal infections (3 of 15; 20%). An association between the AP-PCR genotype and the origin of *A. actinomycetemcomitans* strains was noted when genotype 3 occurred statistically significantly (phi-square = 0.1330; $P = 0.0197$) more frequently in subjects with focal infections (5 of 15; 33%) than in those whose blood samples were tested (2 of 35; 6%) (Table 2). No statistically significant differences in the frequencies of other *A. actinomycetemcomitans* serotypes or genotypes in blood or in focal infections were observed.

Table 3 shows the MICs at which 50% of isolates are inhibited (MIC_{50}s) and the MIC_{90}s of the six antimicrobial agents

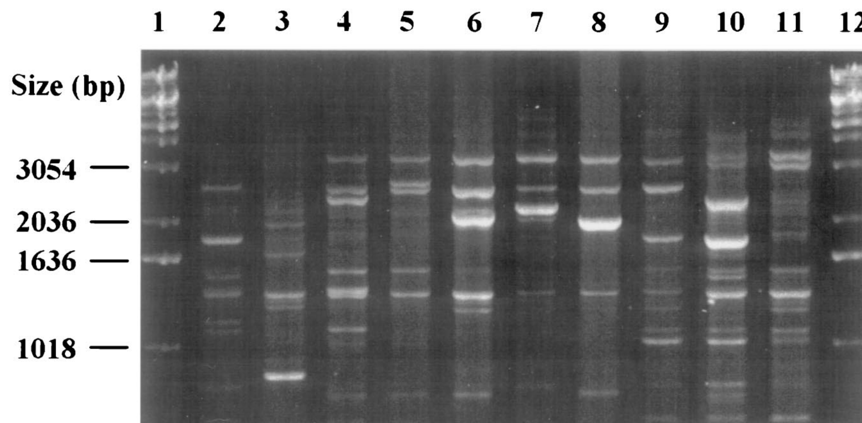

FIG. 1. Ten different AP-PCR banding patterns (lanes 2 to 11) among 52 nonoral *A. actinomycetemcomitans* strains from 51 subjects. Lanes 1 and 12, DNA markers. The banding patterns in lanes 6, 8, 10, and 11 were not previously found in our studies with oral *A. actinomycetemcomitans* isolates (2, 3, 14, 28).

for all 73 *A. actinomycetemcomitans* strains tested. In all tests the MICs for the *Haemophilus* and the other control strains were in acceptable ranges. No differences in the MIC_{50}s or the MIC_{90}s were observed between the nonoral and oral *A. actinomycetemcomitans* strains. Amoxicillin, tetracycline, azithromycin, and trovafloxacin showed good activity against all *A. actinomycetemcomitans* strains, regardless of the infection site. According to the NCCLS breakpoints suggested for *Haemophilus* spp. susceptibility interpretation (26), 21 (29%) of 73 *A. actinomycetemcomitans* strains were resistant to benzylpenicillin, with all strains being of serotype a, b, or c (Table 4). Thus, none of the total of 11 serotype d, serotype e, or nonserotypeable *A. actinomycetemcomitans* strains (nonoral strains, $n = 7$; oral strains, $n = 4$) were resistant to benzylpenicillin.

Table 4 shows that resistance to benzylpenicillin occurred among *A. actinomycetemcomitans* serotype b strains (18 of 29; 62%) statistically significantly (phi-square = 0.3567; P = 0.0000) more frequently than among strains of the other serotypes (3 of 44; 7%), including 11 serotype d, serotype e, or nonserotypeable strains. The same phenomenon was seen for both nonoral (phi-square = 0.3607; P = 0.0000) and oral (phi-square = 0.3471; P = 0.0139) *A. actinomycetemcomitans* strains. Within serotype b, strains of the AP-PCR genotypes 2 and 12 combined together (15 of 18; 83%) exhibited resistance to benzylpenicillin statistically significantly (phi-square = 0.3143; P = 0.0041) more often than strains of the three other serotype b genotypes (3 of 11; 27%). Similarly, strains of the AP-PCR genotypes 2 and 12 combined exhibited resistance to benzylpenicillin statistically significantly (phi-square = 0.4755; P = 0.0000) more frequently than strains of all the other *A. actinomycetemcomitans* genotypes (6 of 55; 11%).

According to the NCCLS breakpoints for anaerobic bacteria (27), 4 (5%) of 73 *A. actinomycetemcomitans* strains were resistant to metronidazole; 2 were of serotype b and two were of serotype c. All four strains had different AP-PCR genotypes.

All 73 nonoral and oral *A. actinomycetemcomitans* strains included in the study displayed the 1,000-bp leukotoxin gene promoter amplicon, whereas reference strain JP2 generated the expected 470-bp amplicon.

TABLE 2. Serotype and AP-PCR genotype[a] distributions among nonoral *A. actinomycetemcomitans* strains from 50 subjects, separately for samples from blood and focal infections, when one strain per subject was analyzed

No. (%) of subjects infected with strains with depicted serotype				No. (%) of subjects infected with strains with depicted AP-PCR genotype within each serotype			
Serotype	Blood	Focal infection	Total	AP-PCR genotype	Blood	Focal infection	Total
a	4 (11)	4 (27)	8 (16)	1	3 (9)	4 (27)	7 (14)
				10	1 (3)	0	1 (2)
b	17 (49)[b]	3 (20)	20 (40)	2	7 (20)	2 (13)	9 (18)
				12	3 (9)	0	3 (6)
				23	7 (20)	1 (7)	8 (16)
c	9 (26)	6 (40)	15 (30)	3	2 (6)	5 (33)[c]	7 (14)
				24	7 (20)	1 (7)	8 (16)
d	2 (6)	2 (13)	4 (8)	22	1 (3)	1 (7)	2 (4)
				25	1 (3)	1 (3)	2 (4)
e	1 (3)	0	1 (2)	24	1 (3)	0	1 (2)
x[d]	2 (6)	0	2 (4)	26	2 (6)	0	2 (4)
Total	35 (100)	15 (100)	50 (100)		35 (100)	15 (100)	50 (100)

[a] AP-PCR genotype designations 1 to 17 are according to Asikainen et al. (3), designations 18 and 19 are according to Paju et al. (28), designations 20 to 22 are according to Dogan et al. (14), and designations 23 to 26 are according to this study.
[b] For frequency of detection of serotype b versus that of the other serotypes in blood samples, P = 0.0553.
[c] For frequency of detection of AP-PCR genotype 3 versus that of the other genotypes in focal infections, P = 0.0197.
[d] *x*, nonserotypeable *A. actinomycetemcomitans* strains.

TABLE 3. MICs of six antimicrobial agents for nonoral and oral *A. actinomycetemcomitans* strains

Antimicrobial agent	MIC (mg/liter)						
	Nonoral *A. actinomycetemcomitans* strains (n = 52)			Oral *A. actinomycetemcomitans* strains (n = 21)			NCCLS breakpoints[a] (mg/liter [susceptible/ resistant])
	Range	50%	90%	Range	50%	90%	
Benzylpenicillin[b]	0.5–8.0	2.0	4.0	1.0–8.0	2.0	4.0	≤1/≥4
Amoxicillin[b]	0.12–2.0	0.5	1.0	0.5–1.0	0.5	1.0	≤1/≥4
Tetracycline	0.25–4.0	0.5	1.0	0.25–1.0	1.0	1.0	≤2/≥8
Azithromycin	0.06–2.0	1.0	1.0	0.25–1.0	1.0	1.0	≤4/—[c]
Trovafloxacin	≤0.06–0.12	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤1/—
Metronidazole[d]	0.25–128.0	16.0	16.0	2.0–128.0	16.0	16.0	≤8/≥32

[a] NCCLS (26, 27) MICs for *Haemophilus* spp. were used.
[b] Breakpoints for ampicillin were used.
[c] —, no breakpoint.
[d] MICs for anaerobic bacteria were used.

DISCUSSION

The study material comprised 52 nonoral *A. actinomycetemcomitans* strains from 51 subjects with various nonoral infections and 21 oral *A. actinomycetemcomitans* strains from 21 subjects. The nonoral strains were collected from distinct geographic locations worldwide, whereas the oral strains originated in our culture collection. Our hypothesis was that the *A. actinomycetemcomitans* strains involved in nonoral infections would represent especially virulent *A. actinomycetemcomitans* clones. Therefore, we compared the frequencies of detection and antimicrobial susceptibilities of *A. actinomycetemcomitans* strains of various serotypes and genotypes obtained from nonoral and oral sampling sites. We additionally analyzed the leukotoxin gene promoter structure in order to find differences between nonoral and oral *A. actinomycetemcomitans* strains.

The serotype and AP-PCR genotype characterizations of the present nonoral *A. actinomycetemcomitans* strains showed wide heterogeneity, with the major serotypes being serotypes a, b, and c (15, 40, and 31% of strains, respectively) and with smaller proportions of serotype d, serotype e, and nonserotypeable strains (8, 2, and 4%, respectively). The AP-PCR technique, a rapid and useful method for the clonal analysis of *A. actinomycetemcomitans* (2, 36), distinguished 10 different genotypes among the present nonoral *A. actinomycetemcomitans* strains. This result shows that a variety of clones may cause nonoral *A. actinomycetemcomitans* infections. The serotype distribution of the present nonoral *A. actinomycetemcomitans* strains resembles that of oral *A. actinomycetemcomitans* strains when all five serotypes are determined (32, 33, 42), supporting the concept that the oral cavity is the ecological niche of *A. actinomycetemcomitans*. Unfortunately, no information on the oral carriage of *A. actinomycetemcomitans* or the periodontal status of the patients who contributed the present nonoral strains was available, and, thus, there is no direct evidence of the plausible sources of the bacterium in these nonoral infections. Nevertheless, the clonal identities of the *A. actinomycetemcomitans* strains recovered from blood and the oral cavity have been confirmed in patients with endarteritis, endocarditis, or bacteremia (24, 30, 39), which suggests that the origin of the present nonoral *A. actinomycetemcomitans* strains was also the human oral cavity.

Four of the AP-PCR genotypes comprising as many as 22 (42%) of all 52 strains were not found in our earlier studies (2, 3, 14, 28). However, since our studies have mainly included oral *A. actinomycetemcomitans* strains from Finnish subjects, the present finding of previously undetectable AP-PCR genotypes hardly suggests specific *A. actinomycetemcomitans* clones in nonoral infections but more likely is due to the widespread geographic origins of the present strains.

Our findings differ from the previous results on the serotype distribution of nonoral *A. actinomycetemcomitans* (45). The data of Zambon and coworkers (45) suggested that serotype c predominates in nonoral infections; 22 (73%) of the 30 *A. actinomycetemcomitans* strains were of serotype c. The num-

TABLE 4. Benzylpenicillin resistance among nonoral and oral *A. actinomycetemcomitans* strains of various AP-PCR genotypes[a] among serotypes a, b, or c

Serotype	Frequency of resistance[b]			AP-PCR genotype	Frequency of resistance[b]		
	Nonoral strains	Oral strains	Total		Nonoral strains	Oral strains	Total
a	1/8 (13)	1/3 (33)	2/11 (18)	1	1/7 (14)	1/3 (33)	2/10 (20)
				10	0/1 (0)	—[c]	0/1 (0)
b	13/21 (62)	5/8 (63)	18/29 (62)	2	7/9 (78)	4/4 (100)	11/13 (85)
				12	3/3 (100)	1/2 (50)	4/5 (80)
				23	3/9 (33)	—	3/9 (33)
				8	—	0/1 (0)	0/1 (0)
				9	—	0/1 (0)	0/1 (0)
c	1/16 (6)	0/6 (0)	1/22 (5)	3	0/8 (0)	0/4 (0)	0/12 (0)
				24	1/8 (13)	—	1/8 (13)
				4	—	0/1 (0)	0/1 (0)
				14	—	0/1 (0)	0/1 (0)

[a] For AP-PCR genotype designations, see footnote *a* of Table 2.
[b] Number of resistant strain(s)/number of strains with the depicted sample origin and serotype or AP-PCR genotype definition (percent).
[c] —, no strains of the depicted AP-PCR genotype were detected.

bers of *A. actinomycetemcomitans* strains included in the present study and in that of Zambon and coworkers (45) are still limited, which, together with the different serotyping methods, may account for the different results between the two studies.

A. actinomycetemcomitans serotype b is strongly associated with periodontal disease (1, 44). In the present study serotype b was the predominant (41% of subjects) serotype in nonoral infections and was more prevalent (49 versus 20%; $P = 0.0553$) (Table 2) in blood samples of endocarditis and bacteremia patients than in focal infections, which are likely less severe than blood infections. This suggests that due to serotype-dependent factors some *A. actinomycetemcomitans* strains may exhibit tropism for certain tissues, such as the endocardium, and may contribute to the course of nonoral infections. The importance of certain *A. actinomycetemcomitans* clones in nonoral infections is further supported by the finding that one *A. actinomycetemcomitans* AP-PCR genotype was significantly ($P = 0.0197$) more frequently found in focal infections than in blood samples. Previously, among a total of 15 distinguishable *A. actinomycetemcomitans* AP-PCR genotypes, strains of this particular AP-PCR genotype were the most frequently detected (32%) in the oral cavities of periodontally healthy subjects (2). Thus, further studies are needed to determine whether certain characteristics enable strains of this genotype to colonize healthy oral cavities and preferentially cause localized nonoral infections.

None of the present oral or nonoral *A. actinomycetemcomitans* strains produced the amplicon characteristic of the deletion of the leukotoxin gene promoter of a highly toxic oral *A. actinomycetemcomitans* strain that is mostly detected among juvenile periodontitis patients of African origin (8, 18). The nonoral *A. actinomycetemcomitans* strains originated from Taiwan, New Zealand, and the United States, but a majority (69%) was received from northern Europe, where the virulent clonal type characterized by high-level production of leukotoxin has not been detected (4, 17). Although the ethnic origin or race of the patients who contributed the nonoral *A. actinomycetemcomitans* strains in the present study were not known, the result supports the current assumption that the deletion of the leukotoxin promoter structure is rare (7).

Amoxicillin, tetracycline, azithromycin, and trovafloxacin exhibited good activities against both nonoral and oral *A. actinomycetemcomitans* strains. However, when applying the NCCLS guidelines (26, 27) in the interpretation of the MIC results, it was seen that approximately 30% of all *A. actinomycetemcomitans* strains, strains of both nonoral and oral origins, were resistant to benzylpenicillin or metronidazole. Our present results largely corroborate those of previous studies from our laboratory and elsewhere on the antimicrobial susceptibilities of oral *A. actinomycetemcomitans* strains (5, 29, 34, 41). *A. actinomycetemcomitans* strains do not produce penicillinase (34); thus, the resistance to benzylpenicillin is probably not beta-lactamase mediated but, instead, may be related to changes in penicillin-binding proteins, as observed among strains of *H. influenzae* (10), a close phylogenetic relative of *A. actinomycetemcomitans*. Interestingly, in the present study serotype b strains were statistically significantly most frequently ($P = 0.0000$; Table 4) resistant to benzylpenicillin, whereas only a few strains of the other serotypes were resistant to benzylpenicillin. Additionally, two AP-PCR genotypes among serotype b strains exhibited benzylpenicillin resistance significantly more often ($P = 0.0041$; Table 4) than the other serotype b genotypes. It is not known whether serotype b strains or, particularly, whether some clones within serotype b have specific properties, such as alterations in penicillin-binding proteins, that would allow them to exhibit increased resistance to benzylpenicillins.

According to the present guidelines of NCCLS (27), two nonoral and two oral *A. actinomycetemcomitans* strains, comprising 5% of all strains tested in the present study, were resistant to metronidazole. As has been shown in earlier studies, resistance to metronidazole occurs among oral *A. actinomycetemcomitans* strains (5, 21, 29, 34, 41), which can be expected due to its oxygen tolerance. Amoxicillin, the currently recommended antimicrobial agent for use as endocarditis prophylaxis in dental procedures (11), showed good activity against all of the present *A. actinomycetemcomitans* strains, regardless of the origin of the infection site, and therefore can be anticipated to be effective as endocarditis prophylaxis for periodontitis patients harboring oral *A. actinomycetemcomitans*. Our results for amoxicillin corroborate previous results for oral *A. actinomycetemcomitans* strains (41). Also, trovafloxacin, a new quinolone, which has excellent activity against several microaerophilic bacterial species (38) but whose activity has not previously been tested against *A. actinomycetemcomitans*, showed high levels of activity against the present strains. However, to date no information on the in vivo efficacy of trovafloxacin against *A. actinomycetemcomitans* infections is available.

In conclusion, the serotype and genotype characteristics of nonoral *A. actinomycetemcomitans* strains highly resembled those of the oral strains and suggest that the origin of the strains was the human oral cavity. The predominance of serotype b strains in nonoral *A. actinomycetemcomitans* infections and the relationship between serotype b strains and bacteremia or endocarditis as well as between certain AP-PCR genotypes and focal infections support the hypothesis that certain *A. actinomycetemcomitans* clones are important contributors to nonoral infections. However, the relatively small sample sizes in the present comparisons provoke the need for additional studies with larger sample sizes to prove the relationship between an *A. actinomycetemcomitans* strain and a specific infection. Additionally, further studies on the exceptional resistance of *A. actinomycetemcomitans* serotype b strains to benzylpenicillin are warranted.

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