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Short communication

# Detection and serotype distribution of *Actinobacillus actinomycetemcomitans* in cardiovascular specimens from Japanese patients

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*Actinobacillus actinomycetemcomitans*, an important pathogen in periodontitis, has also been detected in cardiovascular tissues. Sixty heart valves were collected during valve replacement surgery from 60 patients (one from each), 10 were from patients with infective endocarditis (IE group) and 50 were from patients with other valvular diseases (non-IE group). In addition, 46 samples of aneurysmal tissue were taken from 46 patients with a thoracic or abdominal aneurysm (Aneurysm group, one from each). Dental plaque samples were taken from 54 of the patients, 31 in the IE and non-IE groups and 23 in the aneurysm group. First, the distribution of *A. actinomycetemcomitans* in all specimens was analysed using a polymerase chain reaction method, which resulted in a positive reaction in 33 (31.1%) of the cardiovascular specimens and 25 (46.3%) of the dental plaque samples. Next, using serotype-specific sets of primers, the serotype distribution of *A. actinomycetemcomitans* in the cardiovascular specimens and dental plaque samples was found to be significantly different compared to dental plaque samples from Japanese subjects reported previously.

Key words: *Actinobacillus actinomycetemcomitans*; cardiovascular specimens; polymerase chain reaction; serotype

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*Actinobacillus actinomycetemcomitans*, a gram-negative facultatively anaerobic coccobacillus, is an important pathogen related to periodontitis (2). Recent accumulated evidence suggests that oral bacterial pathogens are associated with several kinds of systemic diseases, such as infective endocarditis and cardiovascular disease (9, 11). These associations are considered to be initiated by transient or prolonged bacteremia caused by oral infection, from such practices as professional dental treatments and daily oral care, i.e. tooth brushing and flossing, and from food chewing, which possibly induces dissemination of oral bacteria into the bloodstream (20). *A. actinomycetemcomitans* has been classified into six serotypes (a–f) based on the immunodominant outer membrane antigen of O-polysaccharide in lipopolysaccharide (6, 17, 18). The serotype distribution was determined by a conventional immunological method using serotype-specific antisera; however, the recent establishment of molecular techniques using the polymerase chain reaction has enabled more rapid and sensitive determination of each serotype (21).

Several studies have focused on the distribution of periodontitis-related bacterial species in cardiovascular specimens. The detection frequency of *A. actinomycetemcomitans* in 50 atheromatous specimens obtained during carotid endarterectomies in the United States was reported to be 18% (4), while another study that analysed the presence of five periodontal species in coronary artery plaque specimens taken from 51 patients in Japan reported frequent (23.3%) identification of *A. actinomycetemcomitans* (5). In addition, a high detection rate (40–56%)

of *A. actinomycetemcomitans* in atheromatous plaque specimens was reported in an analysis of 29 patients in the United States (7).

We analysed cardiovascular specimens collected by the Department of Cardiovascular Surgery at Osaka Rosai Hospital, Sakai, Osaka, Japan, from December 2004 to March 2006, following approval of the study protocol by the Ethics Committee of Osaka Rosai Hospital. Table 1 summarizes the origin of the 106 heart valve and atheromatous plaque specimens (one specimen from each patient), as well as the 54 dental plaque samples taken from 54 of those patients. The heart valve specimens were divided into two groups; those from patients diagnosed with infective endocarditis (IE group) and those with other valvular diseases (non-IE group). In addition, atheromatous plaque specimens were collected from patients undergoing surgery for thoracic or abdominal aneurysms (Aneurysm group). The heart valve and atheromatous plaque specimens were cut into small pieces under aseptic conditions, then whole DNA fractions were extracted using a method described previously (12). Genomic DNA was also extracted from the dental plaque samples, which were collected during an oral examination carried out before the cardiovascular operation, and analysed as described previously (12).

First, we identified specimens containing the DNA of *A. actinomycetemcomitans* using a polymerase chain reaction method with the primers 5′-CTA GGT ATT GCG AAA CAA TTT G-3′ and 5′-CCT GAA ATT AAG CTG GTA ATC-3′, as described previously (3). Next, serotype specification for the *A. actinomycetemcomitans*-positive specimens was performed with a polymerase chain reaction method using serotype specific primer sets (serotype a; 5′-GCA ATG ATG TAT TGT CTT CTT TTG GA-3′ and 5′-CTT CAG TTG AAT GGG GAT TGA CTA AAA C-3′, serotype b; 5′-CGG AAA TGG AAT GCT TGC-3′ and 5′-CTG AGG AAG CCT AGC AAT-3′, serotype c; 5′-AAT GAC TGC TGT CGG AGC-3′ and 5′-CGC TGA AGG TAA TGT CAG-3′, serotype d; 5′-TTA CCA GGT GTC TAG TCG GA-3′ and 5′-GGC TCC TGA CAA CAT TGG AT-3′, serotype e; 5′-CGT AAG CAG AAG AAT AGT AAA CGT-3′ and 5′-AAT AAC GAT GGC ACA TCA GAC TTT-3′, and serotype f; 5′-CCT TTA TCA ATC CAG ACA GC-3′ and 5′-ARA AYT TYT CWT CGG GAA TG-3′), as previously reported (6, 21). The reference strains of *A. actinomycetemcomitans* ATCC29523 (serotype a), ATCC29522 (b), ATCC33384 (c), IDH781 (d), and IDH1705 (e) were kindly provided by Dr Sirkka Asikainen (Institute of Odontology, Division of Oral Microbiology, Umeå University, Sweden).

Among the cardiovascular specimens, five (50%), 16 (32%), and 12 (26%) in the IE, non-IE and Aneurysm groups, respectively, were positive for *A. actinomycetemcomitans* (Table 2). As for the dental plaque samples, two (40%), 12 (46%) and 11 (48%) from those groups, respectively, were positive for *A. actinomycetemcomitans*. An analysis of the serotype distribution of *A. actinomycetemcomitans* in the cardiovascular specimens identified only serotype e, however, most of the specimens could not be classified as any known serotype (a–f). Since most of the *A. actinomycetemcomitans*-positive specimens were not reactive with any of the serotype-specific primer sets, additional evaluations were carried out using an *A. actinomycetemcomitans*-specific set of primers constructed by Conrads et al. (1). All of the untypeable specimens showed a positive reaction to this set of primers, which demonstrated that the present untypeable specimens probably contained DNA of *A. actinomycetemcomitans*.

The serotype distribution of *A. actinomycetemcomitans* in oral isolates has been analysed in several countries. In Japan, an analysis of 156 strains isolated from periodontitis patients using an immunodiffusion method detected serotypes a (26.8%), e (25.5%), and c (24.8%) at high frequencies (22). On the other hand, the serotype distribution in samples from 328 periodontally healthy subjects using the same polymerase chain reaction method as that utilized in the present study revealed that the most prevalent serotype was e (46.7%), followed by serotypes c (41.3%) and d (25.3%) (23). Together, these results suggest that serotype e strains are more prevalent in Japanese subjects than strains with other serotypes regardless of periodontal condition.

In contrast, an analysis of 185 subjects in China including 31 periodontitis patients detected *A. actinomycetemcomitans* in 116, of whom two-thirds had serotype c organisms (10). In addition, that survey also found that the detection rate of serotype e was only 10.9%. In a survey in Finland of 515 isolates from 21 periodontally healthy subjects and 70 subjects with periodontitis, the most prevalent serotype was c (40.8%), followed by a (25.0%) and b (24.7%), with serotype e detected in only 3.5% (19). Together, these findings suggest that the serotype distribution of *A. actinomycetemcomitans* is dependent on geographical location.

To the best of our knowledge, there is only a single report of the serotype distribution of *A. actinomycetemcomitans* in

*Table 1.* Summary of cardiovascular specimens analyzed in this study

| Group | Diagnosis | Total cases | Number of collected specimens | |
|---|---|---|---|---|
| | | | Cardiovascular tissue only | Cardiovascular tissue and dental plaque pair |
| IE (n = 10) | Infective endocarditis | 10 | 5 | 5 |
| Non-IE | Aortic regurgitation | 14 | 5 | 9 |
| (n = 50) | Aortic stenosis | 15 | 8 | 7 |
| | Aortic stenosis & regurgitation | 9 | 7 | 2 |
| | Mitral regurgitation | 9 | 3 | 6 |
| | Mitral stenosis | 1 | 0 | 1 |
| | Tricuspid regurgitation | 2 | 1 | 1 |
| Aneurysm | Thoracic aortic aneurysm | 20 | 10 | 10 |
| (n = 46) | Abdominal aortic aneurysm | 26 | 13 | 13 |

*Table 2.* Detection and serotype distribution of *Actinobacillus actinomycetemcomitans*

| Serotype | Cardiovascular specimens ($n = 106$) | | | Dental plaque ($n = 54$) | | |
|---|---|---|---|---|---|---|
| | IE ($n = 10$) | Non-IE ($n = 50$) | Aneurysm ($n = 46$) | IE ($n = 5$) | Non-IE ($n = 26$) | Aneurysm ($n = 23$) |
| a | 0 | 0 | 0 | 0 | 0 | 0 |
| b | 0 | 0 | 0 | 0 | 0 | 0 |
| c | 0 | 0 | 0 | 0 | 0 | 0 |
| d | 0 | 0 | 0 | 0 | 0 | 0 |
| e | 2 | 3 | 3 | 0 | 3 | 3 |
| f | 0 | 0 | 0 | 0 | 0 | 3 |
| Untypeable | 3 | 13 | 9 | 2 | 9 | 5 |
| | 5 (50%) | 16 (32%) | 12 (26%) | 2 (40%) | 12 (46%) | 11 (48%) |

*Table 3.* Comparison of serotypes of *Actinobacillus actinomycetemcomitans* in tissue–plaque pairs from the same subjects

| Group | Total number of subjects | Number of pairs positive for *A. actinomycetemcomitans* | Serotype of cardiovascular specimen | Serotype of dental plaque specimen | No. of pairs |
|---|---|---|---|---|---|
| IE | 5 | 1 | Untypeable | Untypeable | 1 |
| Non-IE | 26 | 11 | e | e | 3 |
| | | | Untypeable | Untypeable | 8 |
| Aneurysm | 23 | 6 | e | e | 1 |
| | | | Untypeable | e | 1 |
| | | | Untypeable | f | 1 |
| | | | Untypeable | Untypeable | 3 |

blood (15). In that study, 52 *A. actinomycetemcomitans* strains from 51 subjects were analysed and the most common serotypes were found to be serotypes b (40%) and c (31%). Further, the predominance of serotype b strains in subjects with infective endocarditis and bacteremia led the authors to hypothesize a relationship between certain clones and non-oral infection.

A recent *in vitro* analysis of *A. actinomycetemcomitans* showed that serotype b strains had a higher pro-atherogenic potential than serotype d strains (8). In the present study, none of the cardiovascular and dental plaque samples was positive for serotype b (Table 2). Thus, it is important to analyse the *in vitro* properties of serotypes e and f as well as of untypeable strains, because the results may show the virulence of atheromatous plaque formation.

The reasons why there were so many untypeable specimens in our study remain to be elucidated. Untypeable *A. actinomycetemcomitans* strains have been reported to be derived from serotype c isolates and the likelihood of the existence of an additional serotype is small (16). However, that study was performed before serotype f was described and the specimens analysed in the present study came from subjects living in a different region of Japan. Therefore, it might be inappropriate to apply the conclusions of Paju et al. (16) to all Japanese subjects. On the other hand, we speculated that the untypeable specimens in our study belonged to a single novel type or to only a limited number of additional serotypes. Additional analysis is important and may lead to the identification of a novel serotype related to cardiovascular diseases.

*A. actinomycetemcomitans* was detected in both dental plaque and cardiovascular specimens from one, 11, and six subjects in the IE, non-IE and Aneurysm groups, respectively (Table 3). Three dental plaque and cardiovascular specimen pairs from the non-IE group and one pair from the Aneurysm group were serotype e, suggesting that *A. actinomycetemcomitans* in cardiovascular tissue is derived from the oral cavity. In contrast, two of the patients that had serotype e or f in their dental plaque harbored cardiovascular specimens that were untypeable. Identification of multiple serotypes in dental plaque has been reported (19, 23), indicating that untypeable strains exist in both dental plaque and cardiovascular specimens from the same subject, in addition to serotype e or f strains.

The detection rate of *A. actinomycetemcomitans* in the IE group was higher than in the non-IE and Aneurysm groups (50%, 32% and 26%, respectively). In general, streptococci and staphylococci are regarded as the major pathogens of IE, whereas *A. actinomycetemcomitans*, a species of the HACEK group (*Haemophilus* species, *A. actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*), is known to be a pathogen with an extremely low detection rate (11, 13, 14). However, *A. actinomycetemcomitans* was frequently detected in the extirpated heart valve tissues in our study. It is well known that a molecular technique is much more sensitive for the identification of pathogens in blood than a conventional culture method. Whether the high detection frequency in the present study was derived from the application of the more sensitive polymerase chain reaction technique or whether the frequency of IE caused by *A. actinomycetemcomitans* was higher remains to be elucidated. Accumulation of data from additional studies is required to settle this issue.

In summary, we detected *A. actinomycetemcomitans* serotypes e and f as well as untypeable strains in both dental plaque and cardiovascular specimens from Japanese subjects who underwent cardiovascular surgery. The serotype distribution was significantly different from that reported previously in Japan. Further analyses should be carried out with a focus on the relationship between the serotype of *A. actinomycetemcomitans* and its virulence in cardiovascular diseases.

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