Granulicatella adiacens and Abiotrophia defectiva bacteraemia characterized by 16S rRNA gene sequencing

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Traditionally, the identification, epidemiology and spectrum of clinical diseases caused by Granulicatella adiacens and Abiotrophia defectiva are dependent upon their phenotypic characterization. During a 6-year period (July 1995–June 2001), seven and two α -haemolytic streptococci were identified as G. adiacens and A. defectiva, respectively, by 16S rRNA gene sequencing. Three patients with haematological malignancies and neutropenic fever had primary bacteraemia. Three patients with valvular problems or congenital heart disease had infective endocarditis. A patient with ischemic heart disease and cerebrovascular accident had infected aortic atheroma with dissection. A patient with recurrent pyogenic cholangitis had acute cholangitis and a patient with polypoid cystitis and benign prostatic hypertrophy had acute prostatitis. Four of the nine patients died, including all three with G. adiacens infective endocarditis or infected atheroma. For the seven G. adiacens isolates, the API 20 STREP system successfully identified one and five isolates as G. adiacens with >95% and 80-90% confidence, respectively, whereas the Vitek System (GPI) and ATB Expression system (ID32 STREP) successfully identified none and one isolate as G. adiacens. Of the two A. defectiva isolates, none of the three systems successfully identified either of them as A. defectiva. 16S rRNA gene sequencing is the technique of choice for identifying G. adiacens and A. defectiva, and early surgical intervention should be considered when G. adiacens endocarditis is diagnosed.

Received 19 April 2002 Accepted 17 October 2002

INTRODUCTION

Streptococcus adjacens and Streptococcus defectivus were first proposed by Bouvet *et al.* (1989). As a result of 16S rRNA gene sequence data and other phylogenetic analysis, the names Abiotrophia adiacens and Abiotrophia defectiva were proposed by Kawamura *et al.* (1995). A. adiacens was subsequently transferred to Granulicatella adiacens by Collins & Lawson (2000). Traditionally, the identification, epidemiology and the spectrum of clinical diseases caused by G. adiacens and A. defectiva have always been dependent upon phenotypic characterization. It has also been reported that unusual phenotypic characteristics that do not correlate with the species descriptions in the literature are not uncommon (Christensen & Facklam, 2001). In this study, we used 16S rRNA gene sequencing, in combination with traditional phenotypic tests, to define the epidemiology, clinical diseases and outcome of patients with *G. adiacens* and *A. defectiva* bacteraemia. The usefulness of the Vitek, API and ATB Expression systems (all from bioMérieux Vitek), which are commonly used for microbial identification of α haemolytic streptococci in the clinical microbiology laboratory, for identification of *G. adiacens* and *A. defectiva* were also compared.

METHODS

The patients with *G. adiacens* or *A. defectiva* bacteraemia in this study were hospitalized at the Queen Mary Hospital in Hong Kong during a 6-year period (July 1995–June 2001). All clinical data were collected as

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Characteristics of patients with *G. adiacens* and *A. defectiva* bacteraemia are available as supplementary material in JMM Online (http://jmm.sgmjournals.org/).

described by Luk et al. (1998). The BACTEC 9240 blood culture system (Becton Dickinson) was used. In order to identify potential cases of G. adiacens or A. defectiva bacteraemia, in addition to identifying all blood culture isolates by standard conventional biochemical methods (Murray et al., 1999), the Vitek system (GPI, V1305) and the API 20 STREP system version 5.1 were used for species identification of all α haemolytic streptococci other than Streptococcus pneumoniae isolated from blood cultures during the 6-year period. 16S rRNA gene sequencing was performed on all isolates that were identified by both kits as G. adiacens or A. defectiva with $\geq 95\%$ confidence or by either kit as any bacterial species with < 95 % confidence. The ATB Expression system (ID32 STREP, version 1.1) was also used for identification of the nine isolates that were finally defined as G. adiacens (seven isolates) and A. defectiva (two isolates) by 16S rRNA gene sequencing. Antimicrobial susceptibility was determined by E-test for penicillin and Kirby-Bauer disk diffusion method for the other antibiotics using Muller-Hinton agar supplemented with 5 % horse blood and results were interpreted according to the National Committee for Clinical Laboratory Standards criteria for α -haemolytic streptococci. Multiple positive blood cultures with the same isolate obtained from the same patient were counted only once.

Bacterial DNA extraction, PCR and 16S rRNA gene sequencing. Bacterial DNA extraction, PCR amplification and DNA sequencing of the 16S rRNA genes were performed according to Woo et al. (2001a). Primers LPW200 (5'-GAGTTGCGAACGGGTGAG-3') and LPW205 (5'-CTTGTTACGACTTCACCC-3') (Gibco-BRL) were used for the PCR and primers LPW200, LPW205, LPW99 (5'-TTATTGGGCG TAAAGCGA-3') and LPW273 (5'-TTGCGGGACTTAACCCAAC-3') were used for sequencing. The sequence of the PCR products was compared with known 16S rRNA gene sequences in GenBank by multiple sequence alignment using CLUSTAL W (Thompson et al., 1994) and phylogenetic tree construction was performed using the PILEUP method with GROWTREE (Genetics Computer Group).

RESULTS

16S rRNA gene sequencing

Amongst a total of 302 α -haemolytic streptococci other than S. pneumoniae isolated from blood cultures of patients admitted to Queen Mary Hospital during the 6-year period covering July 1995-June 2001, none was identified by both the Vitek system (GPI) and the API system (20 STREP) as G. adiacens or A. defectiva with \geq 95 % confidence. A total of 74 were identified by either kit as any species with < 95%confidence. PCR of the 16S rRNA genes of these isolates showed bands of approximately 1410 bp. Sequencing of the 16S rRNA genes revealed that nine isolates had 16S rRNA genes with >99 % nucleotide identity to that of *G. adiacens* (seven isolates) (GenBank accession no. AB022027) or A. defectiva (two isolates) (D50541) (Fig. 1). G. adiacens and A. defectiva therefore accounted for 2.3 and 0.7 % of bacteraemia due to α -haemolytic streptococci other than S. pneumoniae.

Phenotypic characterization and identification of G. adiacens by commercial systems

Gram staining of all G. adiacens and A. adiacens bacteraemic isolates showed a mixture of Gram-positive and Gramnegative cocci in chains. Variations in the size and morphology of the bacterial cells were also evident. Five of nine





Fig. 1. Phylogenetic tree showing the relationship of the seven G. adiacens and two A. defectiva isolates to related species. The tree was inferred from 16S rRNA sequence data by the neighbour-joining method. Scale bar, estimated distance of 1 substitution per 100 bases using the Jukes-Cantor correction. Names and accession numbers are given as cited in GenBank.

strains (56%) showed satellitism around streaks of Staphylococcus aureus at the time of primary isolation and during the first subculture after primary isolation. For identification of the seven G. adiacens isolates, the API system (20 STREP) successfully identified one (14.3 %) and five (71.4 %) isolates as G. adiacens with >95 % and 80-90 % confidence, respectively. The Vitek system (GPI) did not identify any of the seven isolates as G. adiacens. The ATB Expression system (ID32 STREP) identified one (14.3%) isolate as G. adiacens at 99% confidence. As for the identification of the two A. defectiva isolates, none of the three systems identified these isolates.

Patient characteristics

The median age of the nine patients was 62 (range 15–85). Six (67%) were male and three (33%) were female. All patients had underlying diseases, with cardiovascular diseases in four (44%), haematological malignancies in three (33%), recurrent pyogenic cholangitis in one (11%) and polypoid cystitis and benign prostatic hypertrophy in one (11%) (characteristics of patients are available as supplementary material in JMM Online at http://jmm.sgmjournals. org/). The underlying diseases were all recognized as predisposing factors for the infections of corresponding patients. The three patients with haematological malignancies and neutropenic fever had primary bacteraemia (patients 1, 8 and 9). The three patients with underlying valvular problems or congenital heart disease had infective endocarditis (patients 2, 4 and 7). The patient with ischemic heart disease and cerebrovascular accident had infected aortic atheroma with dissection (patient 6). The patient with recurrent pyogenic cholangitis had acute cholangitis (patient 3). The patient with polypoid cystitis and benign prostatic hypertrophy had acute prostatitis (patient 5). Six (67%) and three (33%), respectively, had community- or hospital-acquired G. adiacens/A. defectiva bacteraemia. Six patients with G. adiacens bacteraemia had G. adiacens recovered in one blood culture, whereas one patient with G. adiacens bacteraemia and the two patients with A. defectiva bacteraemia had the bacteria recovered in multiple blood cultures. Four patients with G. adiacens bacteraemia and the two patients with A. defectiva bacteraemia had the organism as the sole pathogen recovered in their blood cultures whereas, in three patients with G. adiacens bacteraemia, other bacteria were also recovered concomitantly with the G. adiacens (Pseudomonas aeruginosa in patient 1, Klebsiella pneumoniae in patient 3 and Morganella morganii in patient 5). All nine isolates were sensitive to penicillin, cefalothin, clindamycin and vancomycin. Three G. adiacens isolates were resistant to erythromycin (patients 1, 8 and 9). Overall, four patients (44 %) died (patients 1, 4, 6 and 7).

DISCUSSION

Identification of uncommonly encountered bacterial species in clinical microbiology laboratories has always been problematic. Since the number of reference strains used for building databases in commercial kits is usually small for rare species, it is not uncommon to encounter clinical isolates of these species with ambiguous biochemical profiles. Furthermore, even if they are 'successfully' identified by the commercial kits, the low prevalence rate would imply a low positive predictive value. In this study, we describe our experience in using the 'gold standard' of bacterial identification, 16S rRNA gene sequencing, to characterize two rarely encountered bacterial species, *G. adiacens* and *A. defectiva*, recovered from blood cultures of our patients in the past 6 years.

In the present series, the Vitek system (GPI) was not able to identify a single isolate as G. adiacens, whereas the ATB Expression system (ID32 STREP) was only able to identify one of seven G. adiacens isolates as G. adiacens. Although the API system (20 STREP) was able to identify six isolates as G. adiacens, the confidence of identification in five isolates was less than 95%. As for the identification of A. defectiva, all three systems failed to identify either of the two A. defectiva isolates. Therefore, 16S rRNA gene sequencing should be considered the method of choice for identification of these two species. In a recently published study, Casalta et al. (2002) also described the use of 16S rRNA gene sequencing for the identification of Granulicatella elegans from the heart valve of a patient with culture-negative endocarditis. Interestingly, as described in our recent study, the Vitek system (GPI) and the ATB Expression system (ID32 STREP) were again inferior to the API system (20 STREP), in particular for the speciation of β-haemolytic Lancefield group G streptococci (Woo et al., 2001a).

Careful interpretation and reporting of direct Gram-stain results in positive blood cultures are crucial in guiding towards correct microbiological diagnosis and empirical therapy. Recently, we demonstrated using sucrose-supplemented hypertonic blood culture broth that about a quarter of instances of blood-culture-negative neutropenic fever in bone-marrow-transplant recipients (20 cases) that were believed to be of infective origin were due to bacteraemia caused by cell-wall-deficient forms (Woo et al., 2001b). These isolates, recovered as cell-wall-deficient forms only with the help of hypertonic media, showed prompt reversion to the normal forms after subculturing. Unlike the previous 20 cases, in which the cell-wall-deficient forms developed as a result of exposure to antibiotics, the cell-wall-deficient state of G. adiacens and A. defectiva occurs naturally. As a result, the Gram morphology appearance of cell-wall deficiency in G. adiacens and A. defectiva (the present cases recovered in standard blood culture bottles and the former case reported; Bottone et al., 1995) remained the same after repeated subculturing. It had been suggested that this morphological pleomorphism could be due to an unbalanced growth of bacteria related to nutrient limitation (Frehel et al., 1988). The characteristic Gram stain appearance of G. adiacens and A. defectiva in standard blood-culture bottles should raise suspicion of this bacterium for its presumptive microbiological diagnosis.

The epidemiology and spectrum of infections associated with G. adiacens and A. defectiva bacteraemia in this series are similar to those reported in the literature (Christensen & Facklam, 2001). Overall, G. adiacens and A. defectiva accounted for 3 % of bacteraemia due to α -haemolytic streptococcus-related species other than S. pneumoniae. In the present study, the most common causes of G. adiacens and A. defectiva bacteraemia were infective endocarditis/atheroma (44%) and primary bacteraemia in patients with neutropenic fever (33%). This is consistent with a recently reported study, from the Centers for Disease Control and Prevention Streptococcus Laboratory, that used multiple biochemical tests for speciating G. adiacens and A. defectiva (Christensen & Facklam, 2001). In that study, 43 of 76 (57 %) patients with diagnosis given had infective endocarditis, whereas 20 of 76 (26%) patients with diagnosis given had septicaemia/bacteraemia. The predominance of these two diagnoses in patients with G. adiacens and A. defectiva infections has been also described, a phenomenon also observed in other 'viridans streptococci' and compatible with the oral cavity being the reservoir of these bacteria.

G. adiacens endocarditis is associated with high mortality (Bouvet & Acar, 1984). In the present study, all three patients with *G. adiacens* infective endocarditis or infected atheroma died within 48 h after admission. This finding was also observed in an extensive review by Stein & Nelson (1987). In that review, the authors reported that infective endocarditis associated with nutritionally variant streptococci was associated with high bacteriological failure (41 %), a high rate of relapse after therapy (17 %) and high mortality, of 17 %. Early surgical intervention should be considered when *G. adiacens* endocarditis is diagnosed.

The emergence of macrolide resistance among the *G. adiacens* and *A. defectiva* isolates is also of great concern. A

strain of *A. defectiva* was recently reported that was resistant to erythromycin/clindamycin, causing sequential episodes of infective endocarditis in a child (Poyart *et al.*, 2000), of which the mechanism of resistance involved an erythromycinresistance gene *ermB* homologue. In the present series, three of our nine isolates of *G. adiacens* and *A. defectiva* were resistant to erythromycin, clarithromycin and azithromycin. Since macrolides are used as alternatives as prophylaxis for infective endocarditis in patients allergic to penicillin, rising incidence of macrolide resistance may mean failure of this agent in prophylaxis of infective endocarditis in a significant proportion of these patients undergoing dental or other invasive procedures.

ACKNOWLEDGEMENTS

This work was partly supported by the University Development Fund, University Research Grant Council and the Committee of Research and Conference Grants, The University of Hong Kong.

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