SHORT REPORT

Usefulness of the MicroSeq 500 16S rDNA bacterial identification system for identification of anaerobic Gram positive bacilli isolated from blood cultures

S K P Lau, K H L Ng, P C Y Woo, K-t Yip, A M Y Fung, G K S Woo, K-m Chan, T-l Que, K-y Yuen

J Clin Pathol 2006;**59**:219–222. doi: 10.1136/jcp.2004.025247

Using full 16S ribosomal RNA (rRNA) gene sequencing as the gold standard, 20 non-duplicating anaerobic Gram positive bacilli isolated from blood cultures were analysed by the MicroSeq 500 16S rDNA bacterial identification system. The MicroSeq system successfully identified 13 of the 20 isolates. Four and three isolates were misidentified at the genus and species level, respectively. Although the MicroSeq 500 16S rDNA bacterial identification system is better than three commercially available identification systems also evaluated, its database needs to be expanded for accurate identification of anaerobic Gram positive bacilli.

Identification of anaerobic Gram positive bacilli in clinical microbiology laboratories by phenotypic methods is often difficult. Comparison of the gene sequences of bacterial species has shown that the 16S ribosomal RNA (rRNA) gene is highly conserved within a species and among species of the same genus. Hence, it can be used as the new standard for classification and identification of bacteria.[1 2] Recently, we reported the application of this technique for identifying this group of bacteria.[3–7] The MicroSeq 500 16S rDNA bacterial identification system (Perkin-Elmer Applied Biosystems Division, Foster City, California, USA) has been designed for rapid and accurate identification of bacterial pathogens, using the first 527 bp fragment of the 16S rRNA gene. It has been shown that the system is useful for the identification of unusual aerobic pathogenic Gram negative bacilli, coryneform bacteria, mycobacterium, and nocardia species, and various bacterial strains with ambiguous biochemical profiles.[8–12] In our study, we evaluate the usefulness of this system in the identification of 20 non-duplicating anaerobic Gram positive bacilli isolated from blood cultures.

> ''The MicroSeq 500 16S rDNA bacterial identification system is useful for the identification of unusual aerobic pathogenic Gram negative bacilli, coryneform bacteria, mycobacterium, and nocardia species, and various bacterial strains with ambiguous biochemical profiles''

MATERIALS AND METHODS

Bacterial strains

The bacterial strains were isolates from blood cultures of patients hospitalised at the Queen Mary Hospital in Hong Kong during a four year period (January 1998 to December 2001). Isolates were identified as *Clostridium perfringens* and *Propionibacterium acnes* by phenotypic methods. One isolate each of *C perfringens* and *P acnes* and all isolates other than *C perfringens* and *P acne* were subjected to 16S rRNA gene sequencing. One isolate for each species was selected for DNA sequencing of the first 527 bp fragment of the 16S rRNA gene and analysis by the MicroSeq 16S rDNA bacterial identification system, in addition to identification by three commercially available identification systems for anaerobes: the Vitek System (ANI; bioMerieux Vitek, USA, Hazelwood, Missouri, USA), the RapID ANA II system (Innovative Diagnostic Systems, Atlanta, Georgia, USA), and the API system (20A; bioMerieux Vitek). Each isolate was categorised as clinically significant or a contaminant (pseudobacteraemia) by criteria described previously.[13]

Conventional 16S rRNA gene sequencing

Polymerase chain reaction amplification and DNA sequencing of the full 16S rRNA genes were performed according to our previous publications.[3 7 14] Strains 1–13, 15–17, and 20 were amplified with primers LPW58 (5′-AGGCCCGGG AACGTATTCAC-3′) and LPW81 (5′-TGGCGAACGGGTGA GTAA-3′), strains 14 and 19 with primers LPW55 (5′-AGTTTGATCCTGGCTCAG-3′) and LPW325 (5′-CGGATACCTTGTTACGACT-3′), and strain 18 with primers LPW55 (5′-AGTTTGATCCTGGCTCAG-3′) and LPW205 (5′-CTTGTTACGACTTCACCC-3′). The sequences of the polymerase chain reaction products were compared with known 16S rRNA gene sequences in the GenBank by multiple sequence alignment using the CLUSTAL W program.[15]

Identification by the MicroSeq 500 16S rDNA bacterial identification system

Bacterial DNA extracts were amplified with 0.5 μM primers (005F and 531R) according to the manufacturer's instructions. The DNA sequences were analysed using the database provided by the system.

RESULTS

Patient characteristics

Twenty strains, representing 20 non-duplicating anaerobic Gram positive bacilli, were selected for further analysis by the MicroSeq 16S rDNA bacterial identification system and identification by three commercially available identification systems. Table 1 summarises the characteristics of the 20 patients. The clinical details of patients 14 and 18 have been described previously.[3 7]

Conventional 16S ribosomal RNA gene sequencing

Table 1 shows the results of 16S rRNA gene sequence analysis. For all the 20 isolates, there was $< 2\%$ difference between the 16S rRNA gene sequences of the isolates and the most closely matched sequence in the GenBank.

Abbreviation: rRNA, ribosomal RNA

Table 1 Identification of anaerobic Gram positive bacterial isolates by conventional 16S rRNA gene sequencing, commercially available bacterial identification systems, and the Microseq 500 16S rDNA bacterial identification system

	Patient characteristics			Commercially available bacterial identification systems			MicroSeq 500 16S rDNA bacterial identification system	
Patient/ strain no.	Sex/age*	Diagnosis	Conventional 16S rRNA gene sequencing	Vitek ANI	RapID ANA II	API 20A	Identity	% Difference between isolate sequences and closest match
1	M/1 m	Pseudobacteraemia	*Bifidobacterium pseudocatenulatum/ catenulatum*	Unidentified	>99.9% *Clostridium perfringens*	92% *Eubacterium lentum*, 8% *Actinomyces viscosus*	*B catenulatum*	1.4
2	F/94	Pseudobacteraemia	*Clostridium barati*	Unidentified	99.5% *C barati*	68% *Actinomyces israelii*, 17% *Bifidobacterium* sp.	*C barati*	0
3	M/1 m	Necrotising enterocolitis	*Clostridium difficile*	94% *C difficile*	>99.9% *C difficile*	99.9% *C difficile*	*C difficile*	0
4	F/75	Primary bacteraemia	*Clostridium disporicum*	99% *C barati*	>99.9% *C barati*	94% *E lentum*, 3% *A viscosus*	*Clostridium paraputrificum*	3.3
5	F/77	Pseudobacteraemia	*Clostridium indolis*	89% *Clostridium tetani*	73% *Clostridium limosum*, 16% *Clostridium novyi A*, 12% *C tetani*	Unidentified	*Clostridium innocuum*	8.7
6	M/80	Pseudobacteraemia	*C innocuum*	61% *Corynebacterium jeikeium*, 37% *Lactobacillus jensenii*	74% *C innocuum*, 26% *Clostridium subterminale*	98% *C innocuum*	*Eubacterium dolichum*	8.0
7	F/24	Pseudobacteraemia	*Clostridium orbiscindens*	45% *C subterminale*, 39% *Clostriduim histolyticum*, 11% *C jeikeium*	67% *C tetani*, 19% *novyi A*, 12% *E lentum*	94% *E lentum*, 3% *A viscosus*	*Ruminococcus productus*	18.4
8	M/6 m	Intussusception	*C paraputrificum*	81% *Clostridium septicum*	77% *C septicum*, 23% *C paraputrificum*	80% *C paraputrificum*, 19% *C barati*	*C paraputrificum*	0
9	F/44	Acute cholangitis	*Clostridium perfringens*	99% *C perfringens*	>99.9% *C perfringens*	97% *C perfringens*, 3% *Actinomyces naeslundii*	*C perfringens*	0.2
10	M/54	Primary bacteraemia	*Clostridium ramosum*	83% *C barati*, 11% *Lactobacillus catenaforme*	>99.9% *C ramosum*	96% *C ramosum*, 3% *Bifidobacterium* sp.	*C ramosum*	0
11	F/40	Neutropenic fever	*C septicum*	54% *C septicum*, 42% *C paraputrificum*	>99.9% *C septicum*	99.9% *C septicum*	*Clostridium tertium*	2.6
12	F/45	Pseudobacteraemia	*Clostridium sporosphaeroides*	Unidentified	67% *C tetani*, 19% *C novyi A*, 12% *E lentum*	Unidentified	*C sporosphaeroides*	10.6
13	F/78	Pseudobacteraemia	*C tertium*	71% *C tertium*, 22% *Clostridium clostridiforme*	99.9% *C barati*	90% *C tertium*, 7% *Bifidobacterium* sp.	*C tertium*	0
14	F/87	Infected bed sore	*Eggerthella lenta*	83% *C jeikeium*, 7% *Clostridium hastiforme*, 7% *C histolyticum*	>99.9% *E lenta*	92% *E lenta*, 8% *A viscosus*	*E lenta*	0
15	F/85	Primary bacteraemia	*Eubacterium tenue*	81% *Propionibacterium granulosum*, 13% *Actinomyces odontolyticus*	96% *Clostridium sordellii*, 4% *Clostridium bifermentans*	97% *A viscosus*, 2% *E lentum*	*Clostridium tenue*	1.4
16	F/41	Primary bacteraemia	*Lactobacillus casei/ paracasei*	Unidentified	Unidentified	97% *Bifidobacterium* sp., 3% *A israelii*	*L casei/paracasei*	0
17	M/50	Primary bacteraemia	*Lactobacillus rhamnosus*	50% *Lactobacillus jensenii*, 46% *Actinomyces odontolyticus*	>99.9% *Lactobacillus acidophilus*	63% *A naeslundii*, 27% *Lactobacillus acidophilus/ jensenii*	*L rhamnosus*	0
18	M/70	Acute cholecystitis	*Lactobacillus salivarius*	Unidentified	>99.9% *Propionibacterium granulosum*	70% *A naeslundii*, 30% *Bifidobacterium* sp.	*L salivarius*	0.1
19	M/43	Acute cholangitis	*Olsenella uli*	81% *P granulosum*, 11% *Corynebacterium pseudotuberculosis*	Unidentified	43% *Gemella morbillorum*, 37% *Lactobacillus fermentum*, 10% *Propionicum/avidum*	*Atopobium rimae*	11.1
20	F/1 m	Pseudobacteraemia	*Propionibacterium acnes*	99.9% *P acnes*	>99.9% *P acnes*	99.9% *P acnes*	*P acnes*	0

*In years or months (m).

Table 2 Analysis of DNA sequences of strains identified incorrectly using the Microseq 500 16S rDNA bacterial identification system database

		Identification by DNA sequencing of first 527 bp fragment of 16S rRNA gene				
			Analysis using the GenBank database			
Patient/ strain no.	Identification by conventional 16S rRNA gene sequencing	Using MicroSeq 500 16S rDNA database	BM	No. of base (%) difference between strain and BM	2nd BM	No. of base (%) difference between strain and 2nd BM
4	*Clostridium disporicum*	*Clostridium paraputrificum*	*C disporicum*	10 (2.1)	*Clostridium gasigenes*	24 (4.8)
5	*Clostridium indolis*	*Clostridium innocuum*	*C indolis*	14 (2.7)	*Clostridium symbiosum*	45 (8.7)
6	*C innocuum*	*Eubacterium dolichum*	*C innocuum*	9 (1.7)	*Eubacterium cylindroides*	17 (5.4)
7	*Clostridium orbiscindens*	*Ruminococcus productus*	*C orbiscindens*	3 (0.6)	*Bacteroides capillosus*	29 (5.8)
11	*Clostridium septicum*	*Clostridium tertium*	*C septicum*	1 (0.2)	*Clostridium chauvoei*	9 (1.8)
15	*Eubacterium tenue*	*Clostridium tenue*	*Clostridium ghonii*	12 (2.4)	*Clostridium bifermentans*	15 (3.1)
19	*Olsenella uli*	*Atopobium rimae*	*O uli*	0 (0)	*Olsenella profusa*	21 (4.3)

BM, best match.

Identification by the MicroSeq 500 16S rDNA bacterial identification system

The identities of 13 strains were consistent with those obtained by conventional 16S rRNA gene sequencing (table 1). For the remaining seven sequences, four isolates were misidentified at the genus level (strain 6, *C innocuum* misidentified as *Eubacterium dolichum*; strain 7, *C orbiscindens* misidentified as *Ruminococcus productus*; strain 15, *E tenue* misidentified as *C tenue*; and strain 19, *Olsenella uli* misidentified as *Atopobium rimae*), whereas three were misidentified at the species level (strain 4, *C disporicum* misidentified as *C paraputrificum*; strain 5, *C indolis* misidentified as *C innocuum*; and strain 11, *C septicum* misidentified as *C tertium*).

Identification by commercially available bacterial identification systems

The Vitek ANI system was able to identify 10 and four of the 20 isolates, the RapID ANA II system 15 and eight isolates, and the API 20A system nine and nine isolates to the genus and species levels with $>70\%$ confidence, respectively (table 1).

DISCUSSION

Although the MicroSeq 500 16S rDNA bacterial identification system was better than the three commercially available systems in the identification of the 20 anaerobic Gram positive bacilli tested in our present study, its accuracy is still suboptimal. Using conventional 16S rRNA gene sequencing as the gold standard, the MicroSeq 500 16S rRNA bacterial identification system was able to identify 16 of the 20 (80%) isolates to the genus level, and only 13 (65%) of the isolates to the species level in our present study, compared with the corresponding figures of 86.5% and 81.1% in our previous study on bacterial strains of more diverse genera and species,[12] and 97.2% and 89.2% in a study on unusual aerobic Gram negative bacilli.[11]

> "The database of the MicroSeq 500 16S rDNA bacterial identification system needs to be expanded to improve its accuracy in the identification of anaerobic Gram positive bacilli"

The most common reason for the MicroSeq 500 16S rDNA bacterial identification system to fail to identify a bacterium was a lack of the 16S rRNA gene sequence of the particular bacterium in the database, which is in line with results from our previous study.[12] The 16S rRNA gene sequences of five of the misidentified isolates were not included in the system database, probably because they are rarely encountered. When the same 527 bp DNA sequences of these seven misidentified isolates were compared with the known 16S rRNA gene sequences in the GenBank, six yielded the correct identity, with good discrimination between the best and second best match sequences (table 2). Thus, the database of the MicroSeq 500 16S rDNA bacterial identification system needs to be expanded to improve its accuracy in the identification of anaerobic Gram positive bacilli.

Take home messages

- The MicroSeq 500 16S rDNA bacterial identification system identified 13 of 20 non-duplicating anaerobic Gram positive bacilli isolated from blood cultures
- The system compared favourably with three other commercially available identification systems also evaluated
- However, the system's database needs to be expanded for accurate identification of anaerobic Gram positive bacilli

ACKNOWLEDGEMENTS

This work was partly supported by the University Research Grant Council Grant (HKU 7236/02M), and the Committee for Research and Conference Grant, The University of Hong Kong.

Authors' affiliations

S K P Lau, P C Y Woo, A M Y Fung, G K S Woo, K-m Chan, K-Y Yuen, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Pokfulam Road, Hong Kong
K H L Ng, K-t Yip, T-l Que, Department of Clinical Pathology, Tuen Mun Hospital, Hong Kong

Correspondence to: Dr K-Y Yuen, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Pokfulam Road, Hong Kong; hkumicro@hkucc.hku.hk

Accepted for publication 13 April 2005

REFERENCES

1 **Olsen GJ**, Woese CR. Ribosomal RNA: a key to phylogeny. *FASEB J* 1993;**7**:113–23.
2 **Relman DA**, Loutit JS, Schmidt TM, *et al*. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. *N Engl J Med* 1990;**323**:1573–80.

3 **Woo PCY**, Fung AMY, Lau SKP, *et al.* Identification by 16S ribosomal RNA gene sequencing of Lactobacillus salivarius bacteremic cholecystitis. *J Clin Microbiol* 2002;**40**:265–7.
4 **Woo PCY**, Fung AMY, Lau SKP, *et al.* Diagnosis of pelvic actinomycosis by 16S ribosomal RNA gene sequencing and its clinical significance. *Diagn Microbiol Infect Dis* 2002;**43**:113–18.
5 **Woo PCY**, Lau SKP, Woo GKS, *et al.* Bacteremia due to Clostridium hathewayi in a patient with acute appendicitis. *J Clin Microbiol* 2004;**42**:5947–9.
6 **Woo PCY**, Fung AMY, Lau SKP, *et al.* Actinomyces hongkongensis sp. nov. A novel Actinomyces species isolated from a patient with pelvic actinomycosis. *Syst Appl Microbiol* 2003;**26**:518–22.
7 **Lau SKP**, Woo PCY, Woo GKS, *et al.* Eggerthella hongkongensis sp. nov. and Eggerthella sinensis sp. nov. two novel Eggerthella species, account for half of the cases of Eggerthella bacteremia. *Diagn Microbiol Infect Dis* 2004;**49**:255–63.
8 **Cloud JL**, Conville PS, Croft A, *et al.* Evaluation of partial 16S ribosomal DNA sequencing for identification of nocardia species by using the MicroSeq 500 system with an expanded database. *J Clin Microbiol* 2004;**42**:578–84.
9 **Patel JB**, Leonard DG, Pan X, *et al.* Sequence-based identification of Mycobacterium species using the Microseq 500 16S rDNA bacterial identification system. *J Clin Microbiol* 2000;**38**:246–51.
10 **Tang YW**, Von Graevenitz A, Waddington MG, *et al.* Identification of coryneform bacterial isolates by ribosomal DNA sequence analysis. *J Clin Microbiol* 2000;**38**:1676–8.
11 **Tang YW**, Ellis NM, Hopkins MK, *et al.* Comparison of phenotypic and genotypic technique for identification of unusual aerobic pathogenic gram-negative bacilli. *J Clin Microbiol* 1998;**36**:3674–9.
12 **Woo PC**, Ng KH, Lau SK, *et al.* Usefulness of the MicroSeq 500 16S ribosomal DNA-based bacterial identification system for identification of clinically significant bacterial isolates with ambiguous biochemical profiles. *J Clin Microbiol* 2003;**41**:1996–2001.
13 **Weinstein MP**, Towns ML, Quartey SM, *et al.* The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. *Clin Infect Dis* 1997;**24**:584–602.
14 **Lau SKP**, Woo PCY, Tse H, *et al.* Invasive Streptococcus iniae infections outside North America. *J Clin Microbiol* 2003;**41**:1004–9.
15 **Thompson JD**, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;**22**:4673–80.