ORIGINAL ARTICLE

Clostridium bacteraemia characterised by 16S ribosomal RNA gene sequencing

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Background: Owing to problems in accurate species identification of the diverse genus clostridium, the epidemiology and pathogenicity of many species are not fully understood. Moreover, previous studies on clostridium bacteraemia have been limited and relied only on phenotypic species identification.
Aims: To characterise the epidemiology, disease spectrum, and outcome of clostridium bacteraemia using 16S ribosomal RNA (rRNA) gene sequencing.
Method: During a four year period (1998–2001), all cases of clostridium bacteraemia were prospectively studied and all ''non-perfringens'' clostridium isolates identified to the species level by 16S rRNA gene sequencing.
Results: Fifty one blood culture isolates were identified as *Clostridium perfringens* and 17 belonged to 11 other clostridium species. The first case of *C disporicum* infection and two cases of clostridium bacteraemia in children with intussusception were also described. Of the 68 clostridium isolates from 68 different patients, 38 were associated with clinically relevant bacteraemia. The gastrointestinal and hepatobiliary tracts were common sites of both underlying disease and portal of entry in these patients. *Clostridium perfringens* accounted for 79% of all clinically relevant bacteraemia, with the remainder caused by a diversity of species. The attributable mortality rate of clinically relevant clostridium bacteraemia was 29%. Younger age and underlying gastrointestinal/hepatobiliary tract disease were associated with mortality ($p < 0.05$).
Conclusions: Patients with clinically relevant clostridium bacteraemia should be investigated for the presence of underlying disease processes in the gastrointestinal or hepatobiliary tracts. 16S rRNA gene analysis will continue to be useful in further understanding the pathogenicity of various clostridium species.

Clostridium is a heterogeneous genus that consists of over 150 species. In addition to *Clostridium perfringens*, *C difficile*, *C tetani*, *C botulinum*, and *C septicum*, of which the epidemiology and clinical disease spectra are well defined, studies of the pathogenic potential and disease association of the other clostridium species have been hampered by difficulties in accurately identifying these bacteria. For genus identification, spore formation, which is the most distinguishing feature for differentiating clostridium from other genera of anaerobic Gram positive bacilli, is sometimes not obvious in bacterial isolates recovered directly from clinical specimens. For genus and species identification by commercial kits or analysis of cell wall peptidoglyans and metabolic end products by gas chromatography mass spectrometry, the difficulties lie mainly in the limited database compared with the large number of clostridium species and a lack of special equipment and expertise.

> ''We have used 16S rRNA gene sequencing to define the epidemiology, clinical disease spectrum, and outcome of patients with clostridium bacteraemia during a four year period''

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. Thus, it can be used as the new standard for classification and identification of bacteria.[1–3] Although clostridium species are common blood culture isolates, no studies have systematically characterised clostridium bacteraemia by this genotypic identification method. In our study, we have used 16S rRNA gene sequencing to define the epidemiology, clinical disease spectrum, and outcome of patients with clostridium bacteraemia during a four year period.

MATERIALS AND METHODS

Patients and microbiological methods

The bacterial strains used in our study were isolates from blood cultures of patients hospitalised at the Queen Mary Hospital in Hong Kong, China, during a four year period (January 1998 to December 2001). Clinical data were collected prospectively. Clinical specimens were collected and handled according to standard protocols.[4] The BACTEC 9240 blood culture system (Becton Dickinson, Baltimore, Maryland, USA) was used. All anaerobic Gram positive bacilli isolated from blood cultures were identified to the species level. Isolates were identified as *C perfringens* by the presence of double zone haemolysis on blood agar, the ability to produce lecithinase on egg yolk glucose agar, and the Vitek System (ANI; bioMerieux Vitek, Hazlewood, Missouri, USA). Isolates of *Propionebacterium acnes* were identified by their ability to produce catalase and indole and the Vitek System (ANI). All isolates other than *C perfringens* and *P acnes* were subjected to 16S rRNA gene sequencing. All isolates finally identified as clostridium species were included in our study. Each isolate was categorised as clinically relevant or a contaminant (pseudobacteraemia) by clinical and laboratory criteria, as described previously.[5] The criteria include the patient's clinical presentation, physical examination findings, body temperature at the time of the blood culture, leucocyte and differential cell counts, imaging or operative results, histopathological findings, number of positive blood cultures out of the total number performed, and response to

Abbreviations: PCR, polymerase chain reaction; rRNA, ribosomal RNA

Table 1 Identification of the 17 blood culture isolates of non-perfringens clostridium by 16S ribosomal RNA (rRNA) gene sequencing

Isolate	Species identification by 16S rRNA gene sequencing	GB AC of closest match	% Nucleotide identity*
1	*C barati*	X68174	99.8
2	*C difficile*	AF072474	99.9
3	*C disporicum*	Y18176	99.0
4	*C indolis*	AF028351	97.9
5	*C innocuum*	AF028352	99.9
6	*C orbiscindens*	Y18187	100
7	*C paraputrificum*	AB032556	99.4
8	*C paraputrificum*	AB032556	100
9	*C paraputrificum*	AB032556	100
10	*C paraputrificum*	AB032556	99.8
11	*C ramosum*	M23731	99.8
12	*C ramosum*	M23731	99.9
13	*C ramosum*	M23731	99.8
14	*C septicum*	U59278	99.9
15	*C sporosphaeroides*	M59116	98.5
16	*C tertium*	AF227826	99.9
17	*C tertium*	Y18174	100

AC, accession number; GB, GenBank.
*% Nucleotide identity of 16S rRNA gene sequence to the closest match in GB.

treatment. The same isolate recovered from the same patient was counted only once.

Extraction of bacterial DNA for 16S rRNA gene sequencing

Briefly, 80 µl of NaOH (0.05M) was added to 20 µl of bacterial cells suspended in distilled water and the mixture was incubated at 60℃ for 45 minutes, followed by the addition of 6 µl of Tris/HCl (pH 7.0), achieving a final pH of 8.0. The resultant mixture was diluted ×100 and 5 µl of the diluted extract was used for the polymerase chain reaction (PCR).

PCR, gel electrophoresis, and 16S rRNA gene sequencing

Briefly, DNase I treated distilled water and PCR master mix (which contains dNTPs, PCR buffer, and Taq polymerase) were used in all PCR reactions by adding 1 U of DNase I (Pharmacia, Uppsala, Sweden) to 40 µl of distilled water or PCR master mix, incubating the mixture at 25℃ for 15 minutes, and subsequently at 95℃ for 10 minutes to inactivate the DNase I. The bacterial DNA extracts and control were amplified with 0.5µM primers (LPW58, 5′-AGGCCCGGGAACGTATTCAC-3′ and LPW81, 5′-TGGCG AACGGGTGAGTAA-3′; Gibco BRL, Rockville, Maryland, USA). The PCR mixture (50 µl) contained bacterial DNA, PCR buffer (10mM Tris/HCl (pH 8.3), 50mM KCl, 2mM $MgCl_2$, and 0.01% gelatin), 200 µM of each dNTP, and 1.0 U Taq polymerase (Boehringer Mannheim, Mannheim, Germany). The mixtures were amplified by 40 cycles of 94℃ for one minute, 55℃ for one minute, and 72℃ for two minutes, with a final extension at 72℃ for 10 minutes in an automated 0.5 ml GeneAmp PCR system 9700 (Applied Biosystems, Foster City, California, USA). DNase I treated distilled water was used as the negative control. A 10 µl aliquot of each amplified product was electrophoresed in 1.0% (wt/vol) agarose gel, with a molecular size marker (λ DNA AvaII digest; Boehringer Mannheim) in parallel. Electrophoresis in Tris/borate/EDTA buffer was performed at 100 V for 1.5 hours. The gel was stained with ethidium bromide (0.5 µg/ml) for 15 minutes, rinsed, and photographed under ultraviolet light illumination.

The PCR products were gel purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany). Both strands of the PCR products were sequenced twice with an ABI 377 automated sequencer according to the manufacturers' instructions (Perkin-Elmer, Foster City, California, USA) using the PCR primers (LPW58 and LPW81). The sequences of the PCR products were compared with known 16S rRNA gene sequences in the GenBank (http://www.ncbi.nlm.nih.gov) by multiple sequence alignment using the Clustal W program.[6]

Statistical analysis

A comparison of characteristics was made between (1) patients with *C perfringens* and those with non-perfringens clostridium bacteraemia, (2) patients who succumbed and those who survived the clostridium bacteraemia, and (3) patients with clinically relevant clostridium bacteraemia and those with pseudobacteraemia. The χ^2 test was used for categorical variables and the Student's *t* test for age. A p value < 0.05 was regarded as significant.

RESULTS

Identification of clostridium blood culture isolates

In total, 165 anaerobic Gram positive bacilli were isolated from the blood cultures during the four year study period. Of the 165 isolates, 51 were identified as *C perfringens* and 75 as *P acnes* by phenotypic tests. The remaining 39 isolates were subjected to 16S rRNA gene sequencing. PCR of the 16S rRNA genes of these isolates showed bands at about 1200 bp. Sequencing of the 16S rRNA genes revealed that 17 had 16S rRNA genes with $>97\%$ nucleotide identity to that of known non-perfringens clostridium species, indicating that they were non-perfringens clostridium species (table 1). The remaining 22 isolates were identified as non-spore forming anaerobic Gram positive bacilli, and these isolates will not be discussed in the present report. The 68 blood culture isolates of clostridium (51 of *C perfringens* and 17 of non-perfringens clostridium species) were recovered from 68 different patients. Of these 68 isolates, 38 were associated with clinically relevant bacteraemia, whereas the remaining 30 were associated with pseudobacteraemia as a result of contamination. Thirty of the 38 cases of clinically relevant bacteraemia were caused by *C perfringens*, three by *C ramosum* and one by each of *C disporicum*, *C paraputrificum*, *C septicum*, *C tertium*, and *C difficile* (table 2).

Patient characteristics

Tables 2 and 3 tabulate and summarise the characteristics of the 38 patients with clinically relevant clostridium bacteraemia. The incidence of clostridium bacteraemia was similar throughout the four year study period and there was no obvious seasonal variation. The median age was 74 years (range, 1 month to 95 years). Twenty six patients were over 60. The male to female ratio was 23 : 15. Only four patients did not have underlying diseases. The major underlying diseases were gastrointestinal tract disease (11), hepatobiliary tract disease (11), malignancy (11), hypertension (11), diabetes mellitus (seven), cerebrovascular accident (six), immobilisation and/or bed sore (four), and chronic renal failure (three). No definite source of the bacteraemia was identified (primary bacteraemia) in 13 patients, whereas 12 had acute cholecystitis or cholangitis, seven had other intra-abdominal infections, three had infected gangrene of the extremities, two had infected bed sores, and one had pneumonia. Thirty and nine had community and hospital acquired clostridium bacteraemia, respectively. Thirty four patients had clostridium recovered from one blood culture, whereas in four it was recovered from two blood cultures. Nineteen patients had clostridium as the only bacterium recovered from their blood cultures, whereas in the other 19, other bacteria were recovered at the same time as

Table 2 Characteristics of the 38 patients with clinically relevant clostridium bacteraemia

Patient	Sex/Age	Underlying disease	Diagnosis	Blood culture isolate	CA/HA	Positive blood cultures (n)	Other bacteria recovered in blood culture	Outcome
1	F/86	Gallstones	Infected bed sore	*C perfringens*	CA	1	None	Cured
2	F/83	Ca cervix with liver metastasis, gallstones	Primary bacteraemia	*C perfringens*	HA	1	None	Cured
3	M/59	Liver cirrhosis, hepatic encephalopathy	Primary bacteraemia	*C perfringens*	CA	1	None	Died
4	F/44	Carcinoid tumour with liver metastasis	AC	*C perfringens*	CA	1	*Klebsiella pneumoniae*	Died
5	M/92	HT, dementia, gallstones	AC	*C perfringens*	CA	1	*Escherichia coli*	Cured
6	F/77	HT, osteoporosis	Primary bacteraemia	*C perfringens*	CA	1	None	Cured
7	M/1	None	Intussusception	*C perfringens*	CA	1	*E coli, K pneumoniae, Enterococcus gallinarum*	Died
8	M/26	IVDA	Gas gangrene of right leg	*C perfringens*	CA	2	None	Cured
9	F/73	Ca colon, HT, TB	Intra-abdominal abscess	*C perfringens*	CA	1	None	Cured
10	M/79	Cholangiocarcinoma, gallstones	AC	*C perfringens*	CA	1	*Aeromonas sobria*	Cured
11	F/88	Dementia, rectovaginal and vesicovaginal fistula	Infected fistula	*C perfringens*	CA	1	None	Cured
12	M/78	CVA, SSS, RPC	AC	*C perfringens*	CA	2	*Enterobacter aerogenes*	Cured
13	F/75	Gallstones	Acute cholecystitis	*C perfringens*	CA	1	*E coli*	Cured
14	M/69	DM, gallstones, fatty liver, HT, IHD, hyperlipidaemia, PU	Acute cholecystitis	*C perfringens*	CA	1	*E coli*	Cured
15	F/69	RA, Ca cervix, irradiation proctitis, cystitis, hydronephrosis	Primary bacteraemia	*C perfringens*	CA	1	None	Cured
16	M/68	HT, PU	Acute cholecystitis	*C perfringens*	CA	1	*E coli*	Died
17	M/95	Gallstones, CVA	AC and acute cholecystitis	*C perfringens*	CA	1	*E coli*	Cured
18	M/72	DM, HT, PVD, alcoholism	Wet gangrene of ?lower limbs	*C perfringens*	CA	1	*Streptococcus dysgalactiae subsp. dysgalactiae*	Cured
19	M/88	Liver cirrhosis, COPD, HT, DM, CVA, CRF	Primary bacteraemia	*C perfringens*	CA	1	None	Cured
20	M/84	None	AC, subphrenic abscess	*C perfringens*	CA	1	None	Cured
21	F/70	Sjogren's syndrome, osteoporosis, PVD, big toe gangrene, AF	Right foot gangrene	*C perfringens*	HA	1	None	Died
22	M/72	HT, DM, COPD, CRF, RPC, old TB, gallstones	AC	*C perfringens*	CA	1	*E coli, K pneumoniae, Proteus vulgaris*	Died
23	M/64	IHD, DM, HT, colonic diverticulitum	Acute diverticulitis	*C perfringens*	CA	2	*E coli, K pneumoniae*	Cured
24	F/59	RPC	AC	*C perfringens*	CA	1	*E coli*	Cured
25	M/82	Ca pancreas with peritoneal carcinomatosis, GIB, IO	Primary bacteraemia	*C perfringens*	CA	1	None	Cured
26	M/90	None	Aspiration pneumonia	*C perfringens*	CA	1	None	Cured
27	M/46	CRF, liver cirrhosis, hyperparathyroidism	Spontaneous bacterial peritonitis	*C perfringens*	CA	1	None	Died
28	F/89	IHD, CVA, AF, bronchiectasis, pyometra	Primary bacteraemia	*C perfringens*	CA	1	None	Cured
29	M/46	Renal cell Ca with lung and peritoneal metastasis, GIB	Primary bacteraemia	*C perfringens*	HA	1	*E coli, K pneumoniae*	Died
30	F/84	DM, HT, gallstones	Acute cholecystitis	*C perfringens*	CA	1	*E coli, K pneumoniae*	Cured
31	M/54	HCC, history of liver abscess, GIB	Primary bacteraemia	*C ramosum*	CA	1	*Bacteroides fragilis* group	Died
32	M/71	CVA	Infected bed sore	*C ramosum*	HA	1	*Proteus mirabilis*	Cured
33	M/78	Ca sigmoid with liver metastasis	Primary bacteraemia	*C ramosum*	HA	1	*E coli, Pseudomonas aeruginosa, Enterococcus avium*	Died
34	F/75	Dementia, asthma, HT, DM, uterine prolapse	Primary bacteraemia	*C disporicum*	HA	1	None	Cured
35	M/6m	None	Intussusception with peritonitis	*C paraputrificum*	CA	1	None	Cured
36	F/40	AML, dental caries, neutropenic fever	Primary bacteraemia	*C septicum*	CA	1	None	Cured
37	F/12	ALL, mucositis, neutropenic fever	Primary bacteraemia	*C tertium*	HA	2	None	Cured
38	M/1m	Prematurity	Necrotising enterocolitis	*C difficile*	HA	1	*Enterobacter cloacae*	Died

AC, acute cholangitis; AF, atrial fibrillation; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; Ca, carcinoma; CA, community acquired; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure; CVA, cerebrovascular accident; DM, diabetes mellitus; F, female; GIB, gastrointestinal bleeding; HA, hospital acquired; HCC, hepatocellular carcinoma; HT, hypertension; IHD, ischaemic heart disease; IO, intestinal obstruction; IVDA, intravenous drug abuse; M, male; PU, peptic ulcer; PVD, peripheral vascular disease; RA, rheumatoid arthritis; RPC, recurrent pyogenic cholangitis; SSS, sick sinus syndrome; TB, tuberculosis.

Table 3 Summary of characteristics of patients with clinically relevant clostridium bacteraemia

Characteristics	Number of patients (%)
Year	
1998	13 (34)
1999	10 (26)
2000	8 (22)
2001	7 (18)
Month	
January	6 (16)
February	3 (8)
March	2 (5)
April	4 (11)
May	3 (8)
June	4 (11)
July	7 (18)
August	0 (0)
September	3 (8)
October	2 (5)
November	1 (3)
December	3 (8)
Mean age (SD, median, range; in years)	62 (26.3, 74, 1 month to 95)
0–10	3 (8)
11–20	1 (3)
21–30	1 (3)
31–40	0 (0)
41–50	4 (11)
51–60	3 (8)
61–70	4 (11)
71–80	11 (29)
81–90	9 (24)
91–100	2 (5)
Sex (male:female)	23:15
Underlying disease*	
Gastrointestinal tract disease	11 (29)
Hepatobiliary tract disease	11 (29)
Malignancy	11 (29)
Hypertension	11 (29)
Diabetes mellitus	7 (18)
Cerebrovascular accident	6 (16)
Immobilisation and/or bed sore	4 (11)
Chronic renal failure	3 (8)
None	4 (11)
Diagnosis	
Primary bacteraemia	13 (34)
Cholecystitis/cholangitis	12 (32)
Other intra-abdominal infection	7 (18)
Infected gangrene of extremities	3 (8)
Infected bed sore	2 (5)
Pneumonia	1 (3)
Community/hospital acquired	
Community	30 (79)
Hospital	8 (21)
Number of positive blood cultures	
1	34 (89)
2	4 (11)
Monomicrobial/polymicrobial bacteraemia	
Monomicrobial	19 (50)
Polymicrobial	19 (50)
Mortality	11 (29)

*The percentages add up to more than 100% because some patients had more than one underlying disease.

clostridium, with *Escherichia coli* isolated in 12 cases, *Klebsiella pneumoniae* in six, *Aeromonas sobria*, *Bacteroides fragilis* group, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus avium*, *Enterococcus gallinarum*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and Lancefield group G beta-haemolytic *Streptococcus dysgalactiae* subspecies *dysgalactiae* each in one. Overall, 11 patients died.

Table 4 compares and summarises the characteristics of patients with *C perfringens* and those with non-perfringens clostridium bacteraemia. Older age, a diagnosis of cholecystitis/cholangitis, and community acquired infections were associated with *C perfringens* bacteraemia ($p < 0.05$, $p < 0.05$, and $p < 0.005$, respectively).

Table 5 compares and summarises the characteristics of patients who succumbed and those who survived the clostridium bacteraemia. Younger age and underlying gastrointestinal/hepatobiliary tract disease were associated with mortality ($p < 0.05$ in all three comparisons).

Table 6 compares and summarises the characteristics of those patients with clinically relevant clostridium bacteraemia and those with pseudobacteraemia. Underlying hepatobiliary tract disease was associated with clinically relevant bacteraemia ($p < 0.01$), whereas cerebrovascular accident and immobilisation and/or bed sore were associated with pseudobacteraemia ($p < 0.05$ and $p < 0.005$, respectively).

DISCUSSION

In our study, we defined the epidemiology, clinical spectrum, and outcome of clostridium bacteraemia with the aid of 16S rRNA gene sequencing. Most patients were old, and there was a male predominance. Almost all patients had underlying diseases, with gastrointestinal and hepatobiliary tract disease, malignancy, and hypertension being the most frequent. As in previous reports,[7–9] clostridium bacteraemia was primarily community acquired (30 of 38) and often polymicrobial, with other endogenous bacteria concomitantly being isolated (15 of 30). Among the 25 patients with documented foci of infection, the hepatobiliary and gastrointestinal tracts were the major portals of entry, because cholecystitis/cholangitis (eight patients) and intra-abdominal infections (five patients) made up most of the diagnoses. Therefore, patients with clinically relevant clostridium bacteraemia should be investigated for the presence of underlying disease processes in the hepatobiliary or gastrointestinal tracts. Although the gastrointestinal tract was also the major site of entry in previous studies,[8 9] the reported incidences of both underlying hepatobiliary tract disease and cholecystitis/cholangitis were much lower than those reported in our series. We speculate that this is attributable to our high prevalence of ductal stones, clonorchiasis, and recurrent pyogenic cholangitis.[10] Similar to recent studies (77%,[7] 21.7%,[9] and 42%[8]), *C perfringens* was the most common clostridium species associated with bacteraemia (30 of 38; 79%). However, the species responsible for the remainder of the cases were diverse and varied among different studies. Compared with *C perfringens* bacteraemia, non-perfringens clostridium bacteraemia affected younger patients without biliary tract disease and was more frequently hospital acquired.

> "The association between younger age and mortality is probably the result of underlying diseases, because nine of the 11 patients who died had either gastrointestinal or hepatobiliary tract diseases"

Clostridium bacteraemia, when it occurs in immunocompromised hosts, especially those with underlying gastrointestinal or hepatobiliary tract disease, should be managed cautiously. Although various studies have failed to demonstrate the effect of appropriate antimicrobial treatment on the outcome of clostridium bacteraemia,[7 8] the clinical relevance of each episode should be carefully evaluated and appropriate treatment initiated in relevant cases. In patients with underlying hepatobiliary tract disease, the isolation of clostridium from blood cultures is often clinically relevant. However, in those with an underlying cerebrovascular accident or longterm immobilisation, the chance of encountering pseudobacteraemia is high, probably because of the difficulties in venipuncture and the frequent use of femoral veins. The mortality rate in clostridium bacteraemia was high (29%). Younger age and underlying hepatobiliary and gastrointestinal tract disease were associated with increased risk of death. The association between younger age and mortality is

Table 4 Comparison of characteristics of patients with *Clostridium perfringens* and those with non-perfringens clostridium bacteraemia

Characteristics of blood culture isolate	Number of patients (%)		p Value
	Clostridium perfringens (n = 30)	Other clostridium species (n = 8)	
Sex			NS
Male	18 (60)	5 (63)	
Female	12 (40)	3 (38)	
Mean age (SD)	70 (20.8)	41 (33.2)	<0.05
Underlying diseases*			
Gastrointestinal tract disease	8 (27)	3 (38)	NS
Hepatobiliary tract disease	9 (30)	2 (25)	NS
Malignancy	7 (23)	4 (50)	NS
Hypertension	10 (33)	1 (13)	NS
Diabetes mellitus	6 (20)	1 (13)	NS
Cerebrovascular accident	5 (17)	1 (13)	NS
Immobilisation and/or bed sore	3 (10)	1 (13)	NS
Chronic renal failure	3 (10)	0 (0)	NS
Diagnosis			
Primary bacteraemia	8 (27)	5 (63)	NS
Cholecystitis/cholangitis	12 (40)	0 (0)	<0.05
Other intra-abdominal infection	5 (17)	2 (25)	NS
Infected gangrene of extremities	3 (10)	0 (0)	NS
Infected bed sore	1 (3)	1 (13)	NS
Pneumonia	1 (3)	0 (0)	NS
Community/hospital acquired			
Community	27 (90)	3 (38)	
Hospital	3 (10)	5 (63)	<0.005
Number of positive blood cultures			
1	27 (90)	7 (88)	NS
2	3 (10)	1 (13)	
Mono/polymicrobial bacteraemia			
Monomicrobial	15 (50)	4 (50)	NS
Polymicrobial	15 (50)	4 (50)	NS
Mortality	8 (26)	3 (38)	NS

*The percentages add up to more than 100% because some patients had more than one underlying disease.
NS, not significant.

Table 5 Comparison of characteristics of patients who died of and those who survived clostridium bacteraemia

Characteristics	Number of patients (%)		p Value
	Died (n = 11)	Survived (n = 27)	
Sex			
Male	9 (82)	14 (52)	NS
Female	2 (18)	13 (48)	
Mean age (SD)	49 (26.5)	70 (23.9)	<0.05
Underlying diseases*			
Gastrointestinal tract disease	6 (55)	5 (19)	<0.05
Hepatobiliary tract disease	6 (55)	5 (19)	<0.05
Malignancy	4 (36)	7 (26)	NS
Hypertension	2 (18)	9 (33)	NS
Diabetes mellitus	1 (9)	6 (22)	NS
Cerebrovascular accident	1 (9)	5 (19)	NS
Immobilisation and/or bed sore	1 (9)	3 (11)	NS
Chronic renal failure	2 (18)	1 (4)	NS
Diagnosis			
Primary bacteraemia	4 (36)	9 (33)	NS
Cholecystitis/cholangitis	3 (27)	9 (33)	NS
Other intra-abdominal infection	3 (27)	4 (15)	NS
Infected gangrene of extremities	1 (9)	2 (7)	NS
Infected bed sore	0 (0)	2 (7)	NS
Pneumonia	0 (0)	1 (4)	NS
Community/hospital acquired			
Community	7 (64)	23 (85)	NS
Hospital	4 (36)	4 (15)	
Number of positive blood cultures			
1	11 (100)	23 (85)	NS
2	0 (0)	4 (15)	
Mono/polymicrobial bacteraemia			
Monomicrobial	3 (27)	16 (59)	NS
Polymicrobial	8 (73)	11 (41)	NS

*The percentages add up to more than 100% because some patients had more than one underlying disease.
NS, not significant.

Table 6 Comparison of characteristics of patients with clinically relevant clostridium bacteraemia and those with clostridium pseudobacteraemia

Characteristics	Number of patients (%)		
	Clinically relevant bacteraemia (n = 38)	Pseudobacteraemia (n = 30)	p Value
Sex			
Male	23 (61)	13 (43)	NS
Female	15 (39)	17 (57)	
Mean age (SD)	64 (26.3)	70 (21.6)	NS
Underlying disease*			
Gastrointestinal tract disease	11 (29)	7 (23)	NS
Hepatobiliary tract disease	11 (29)	1 (3)	<0.01
Malignancy	11 (29)	6 (20)	NS
Hypertension	11 (29)	10 (33)	NS
Diabetes mellitus	7 (18)	6 (20)	NS
Cerebrovascular accident	6 (16)	13 (43)	<0.05
Immobilisation and/or bed sore	4 (11)	16 (53)	<0.005
Chronic renal failure	3 (8)	0 (0)	NS
Blood culture isolate			
C barati	0 (0)	1 (3)	NS
C difficile	1 (3)	0 (0)	NS
C disporicum	1 (3)	0 (0)	NS
C indolis	0 (0)	1 (3)	NS
C innocuum	0 (0)	1 (3)	NS
C orbiscindens	0 (0)	1 (3)	NS
C paraputrificum	1 (3)	3 (10)	NS
C perfringens	30 (79)	21 (70)	NS
C ramosum	3 (8)	0 (0)	NS
C septicum	1 (3)	0 (0)	NS
C sporosphaeroides	0 (0)	1 (3)	NS
C tertium	1 (3)	1 (3)	NS
Community/hospital acquired			
Community	30 (79)	21 (70)	NS
Hospital	8 (21)	9 (30)	
Number of positive blood cultures			
1	34 (89)	30 (100)	NS
2	4 (11)	0 (0)	
Mono/polymicrobial bacteraemia			
Monomicrobial	19 (50)	16 (53)	NS
Polymicrobial	19 (50)	14 (47)	NS

*The percentages add up to more than 100% because some patients had more than one underlying disease.
NS, not significant.

probably the result of underlying diseases, because nine of the 11 patients who died had either gastrointestinal or hepatobiliary tract diseases. This is in line with a previous study from Taiwan in which younger age and underlying liver cirrhosis were also shown to be associated with fatal clostridium bacteraemia, although younger age was not an independent risk factor in multivariate analysis.[7] In a previous study on *E coli* bacteraemia, mortality was found to be higher in patients with polymicrobial rather than monomicrobial bacteraemia.[11] There was also a trend for such an association in our present study, but the difference was not significant. Patients with risk factors for higher mortality should be carefully treated with antibiotics and the source promptly identified.

With the application of 16S rRNA gene sequencing, the present report also described the first case of *C disporicum* infection in humans, and two cases of clostridium bacteraemia in children presenting with intussusception. *Clostridium disporicum* is a saccharolytic clostridium species first isolated from the caecum of a rat.[12] It was previously regarded as a non-pathogenic clostridium species, because isolation of this organism from humans has not been reported in the literature. Our present patient (patient 34) with *C disporicum* bacteraemia was admitted for uterine prolapse with ring pessary inserted, and developed fever with neutrophilia after admission. Although the source of the bacteraemia could not be ascertained, the isolation of this bacterium suggests that it is a potential human pathogen. As for the association between intussusception in paediatric patients with clostridium infections, to the best of our knowledge, the only case documented in the English literature was a 19 month old girl with ileocolic intussusception associated with *C difficile* enterocolitis,[13] (Medline search up to August 2004), whereas intussusception in our two infants was associated with *C perfringens* (patient 7) and *C paraputrificum* (patient 35).

Take home messages

- 16S rRNA gene analysis was useful for investigating clostridium bacteraemia
- *Clostridium perfringens* accounted for 79% of all clinically relevant bacteraemia, with the remainder caused by a diversity of species
- The attributable mortality rate of clinically relevant clostridium bacteraemia was 29%
- Younger age and underlying gastrointestinal/hepatobiliary tract disease were associated with mortality and patients with clinically relevant clostridium bacteraemia should be investigated for the presence of underlying disease processes in the gastrointestinal or hepatobiliary tracts
- 16S rRNA gene analysis will continue to be useful in further understanding the pathogenicity of various clostridium species

Patient 7, who had polymicrobial *C perfringens* bacteraemia after successful hydrostatic reduction, developed sudden cardiac arrest and died on the day of admission, with the same blood culture isolates recovered in postmortem tissues of the terminal ileum and mesenteric lymph nodes. Patient 35, who had monomicrobial *C paraputrificum* bacteraemia, presented with peritonitis and septic shock as a result of small bowel ischaemia. He was cured after small bowel resection and appropriate antibiotic treatment. In both cases, there was probably bacterial translocation through the inflamed intestinal mucosa, and the serious outcome suggests that clostridium bacteraemia in patients with intussusception may be a poor prognostic indicator.

The identification and classification of clostridium are difficult because the genus is one of the most heterogeneous bacterial genera and comprises more than 150 species. With the availability of molecular techniques, most clostridium species have been subjected to 16S rRNA gene sequence analysis, and phylogenetic clusters have been defined and many revisions made in their classification.[14–18] The application of these techniques to identify clinically important clostridium isolates will better define the epidemiology, clinical relevance, and pathogenic potential of various species in the genus. Previous reports on clostridium bacteraemia have relied on phenotypic identification of the blood culture isolates, some of which may have been misidentified, and rarely encountered species may have been missed. Large numbers were reported as clostridium species that could not be identified further (35%[8] and 13%[9]). Given the diversity of the genus, it is unlikely that commercially available identification kits will include all clostridium species in their databases. As PCR and sequencing techniques are becoming more readily available in clinical laboratories, 16S rRNA gene analysis is probably the most practical approach to identify members of the genus clostridium to the species level.

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