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Identification and Antimicrobial Resistance Patterns of Clinical Isolates of *Clostridium clostridioforme*, *Clostridium innocuum*, and *Clostridium ramosum* Compared with Those of Clinical Isolates of *Clostridium perfringens*

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***Clostridium ramosum*, *C. innocuum*, and *C. clostridioforme* are frequently isolated from clinical specimens including blood. Because of Gram stain variability, a lack of spores, and atypical colonial morphology, identification of these species is often difficult. Three anaerobe identification kits were evaluated for their abilities to identify these species. For comparison, 11 strains of *C. perfringens* were evaluated in parallel. By using profile numbers and codebooks, the correct genus and species were identified, as follows: with the RapID ANA II kit, 100% (20 of 20) of *C. ramosum* isolates, 24% (5 of 21) of *C. innocuum* isolates, and 50% (10 of 20) of *C. clostridioforme* isolates; with the AnIDent kit, 60% (12 of 20) of *C. ramosum* isolates, 28% (6 of 21) of *C. innocuum* isolates, and 90% (18 of 20) of *C. clostridioforme* isolates; with the ATB32A kit, 70% (14 of 20) of *C. ramosum* isolates, 0% (0 of 21) of *C. innocuum* isolates, and 40% (8 of 20) of *C. clostridioforme* isolates. Profile numbers that overlapped several species were obtained as follows: with the RapID ANA II kit, 0% of *C. ramosum* isolates, 76% of *C. innocuum* isolates, and 40% of *C. clostridioforme* isolates; with the AnIDent kit 40% of *C. ramosum* isolates, 62% of *C. innocuum* isolates, and 5% of *C. clostridioforme* isolates; with the ATB32A kit, 15% of *C. ramosum* isolates, 52% of *C. innocuum* isolates, and 25% of *C. clostridioforme* isolates. One strain of *C. innocuum* was misidentified by the AnIDent kit, and the remainder yielded profile numbers that were not listed in the codebooks. The MICs of 11 antimicrobial agents including penicillin G, metronidazole, clindamycin, cefoxitin, cefotetan, imipenem, meropenem, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, and vancomycin were determined by the agar dilution method. All *C. perfringens* strains were susceptible to all antimicrobial agents tested. Various levels of resistance to cefoxitin, cefotetan, and penicillin G were noted with *C. ramosum*, *C. clostridioforme*, and *C. innocuum*. In addition, resistance to clindamycin was noted with *C. ramosum* (5%) and *C. innocuum* (10%). Most strains of *C. innocuum* were only moderately susceptible to vancomycin (MIC at which 90% of strains are inhibited, 4 μg/ml).**

Members of the genus *Clostridium* form an important part of the anaerobic microflora of humans, with the potential for causing both endogenous and exogenous infections. Apart from the classical clostridial diseases of tetanus and gas gangrene, *Clostridium* species may be present in a variety of clinical specimens, for which the pathogenic potential of a given isolate is frequently determined by its identification. Because any clostridial isolate from a blood culture must be considered potentially significant, prompt and accurate identification is required. Whereas simple techniques are used for the confirmation of the more common clostridial species, identification of other species such as *Clostridium ramosum*, *C. innocuum*, and *C. clostridioforme*, the so-called RIC group, can pose a problem for the routine diagnostic laboratory. This group can easily be misidentified as belonging to other genera because of factors such as Gram stain variability, lack of spores, and atypical clostridial colonial morphology. Experiences at the Anaerobe Reference Unit (Public Health Service Laboratory, Cardiff, United Kingdom) suggest problems in the identification of this group of clostridia, as indicated by the number of referrals for definitive identification (2a). The referring laboratories generally use commercial anaerobe kits to identify their isolates. Although miniaturization of identification systems that provide results within 4 h and the use of non-growth-dependent reactions make these systems practical alternatives for the identification of many anaerobes in clinical laboratories, previous evaluations of these systems report problems with the identification of some anaerobes including certain *Clostridium* species (3, 4, 7, 9). Previous studies (5, 12, 14, 15) also suggest that the RIC group of clostridia display greater levels of resistance to antibiotics compared with *C. perfringens*. Thus, problems in identifying the RIC group should be resolved so that appropriate antibiotic therapy may be selected.

We studied the abilities of three currently available anaerobe identification kits to identify the RIC clostridia. In addition, the MICs of 11 antimicrobial agents were determined by the agar dilution technique.

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MATERIALS AND METHODS

Clinical isolates and control strains. Twenty clinical isolates of *C. ramosum*, 21 clinical isolates of *C. innocuum*, and 20 clinical isolates of *C. clostridioforme*

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were evaluated in the present studies. In addition, 11 strains of *C. perfringens* were included for comparative purposes. Clinical isolates were obtained from the R. M. Alden Culture Collection (Santa Monica, Calif.) and from the Anaerobe Reference Unit. Fourteen of 20 (60%) *C. ramosum* strains, 8 of 20 (40%) *C. innocuum* strains, 8 of 21 (38%) *C. clostridioforme* strains, and 4 of 11 *C. perfringens* strains were originally isolated from blood cultures; the remaining strains were from intra-abdominal and soft tissue infections. All isolates had previously been identified by standard methods, including analysis with prereduced anaerobically sterilized (PRAS) biochemicals (Carr Scarborough Microbiologicals, Inc., Atlanta, Ga.) (6, 13). Control strains included *Bacteroides fragilis* ATCC 25285, *C. ramosum* ATCC 25582, *C. innocuum* ATCC 14501, *C. clostridioforme* ATCC 25537, and *C. perfringens* ATCC 13124.

Identification. The anaerobe identification kits evaluated included RapID ANA II (Innovative Diagnostic Systems, Norcross, Ga.) and AnIDent and ATB 32A (BioMerieux, Hazlewood, Mo.). The RapID ANA II system has 10 wells, 8 of which are bifunctional, for the detection of 18 preformed enzymes. The AnIDent system has two rows of 10 microcupules containing dehydrated substrates for the detection of preformed enzymes plus catalase. The ATB 32A system is a 32-well system that detects 27 preformed enzymes plus the fermentation of mannose and raffinose. Although the ATB 32A system is not approved by the U.S. Food and Drug Administration for human clinical use in the United States, it is used for nonclinical studies and is also used extensively for clinical studies in Europe. The specific test reactions contained in each of the anaerobe identification systems are compared in Table 1.

Each isolate was subcultured onto brucella blood agar (BBA) plates supplemented with vitamin K and hemin (Anaerobe Systems, San Jose, Calif.), and the plates were incubated for 48 h at 35°C under anaerobic conditions. Cell paste was harvested and suspended in water or buffer, provided by the manufacturer, and the turbidity was matched to the appropriate McFarland standard according to the manufacturers' instructions. Each isolate was inoculated into each of the anaerobe identification systems and was incubated aerobically for 4 h at 36°C. A numerical code was generated from each of the test results and was matched to the profiles in the kits' respective codebooks. Identifications were recorded as correct to the genus and species levels, correct to the genus level with a low selectivity for the species level, incorrect genus or species, or no identification. Manufacturers were not called for the identification of isolates with profile numbers not listed in the codebooks. For selected isolates, the tests were repeated with cultures grown on supplemented Columbia and brain heart infusion agars (Hardy Diagnostics Inc., Santa Maria, Calif.).

For isolates identified as an incorrect species, the original identification was confirmed by the use of PRAS biochemicals and the sugar plate method of Rotimi et al. (11) as used by the Anaerobe Reference Unit of the Public Health Laboratory Service of England and Wales. In addition, gas-liquid chromatographic analysis of volatile fatty acid metabolites was performed on 48-h cultures of cooked meat broths as described previously (6, 13) by using a gas chromatograph (Pye Unicam Series 204) fitted with a flame ionization detector and a 10% FFAP column at 150°C. Volatile fatty acid peaks were identified by comparison of the retention times with those of known volatile fatty acids in a standard solution.

Antimicrobial susceptibility testing. The MICs of 11 antimicrobial agents were determined by the Wadsworth agar dilution method (10, 13). The following standard laboratory powders were obtained from the indicated sources: clindamycin (The Upjohn Co., Kalamazoo, Mich.), metronidazole (G. D. Searle & Co., Skokie, Ill.), penicillin G (Sigma Chemical Company, St. Louis, Mo.), vancomycin (Eli Lilly & Co., Indianapolis, Ind.), cefoxitin and imipenem (Merck & Co., Rathway, N.J.), meropenem and cefotetan (Zeneca Inc., Wilmington, Del.), ampicillin-sulbactam (Pfizer, Roerig Division, Groton, Conn.), amoxicillin-clavulanate (SmithKline Beecham Laboratories, Philadelphia, Pa.), and piperacillin-tazobactam (Lederle Laboratories, Pearl River, N.Y.). The antimicrobial agents were reconstituted according to the manufacturers' instructions. Serial twofold dilutions of the antimicrobial agents were prepared and added to molten brucella agar supplemented with vitamin K_1, hemin, and 5% laked sheep blood. Plates were prepared on the day of the test.

Each inoculum was prepared in the anaerobic chamber by harvesting cell paste from 48-h BBA plates and suspending it in brucella broth to a turbidity equivalent to that of a 0.5 McFarland standard. The concentration was verified for selected isolates by quantitative plating. The inocula were applied to the antibiotic-containing plates with a Steers-type replicator (Craft Machine Inc., Chester, Pa.) that delivered a final concentration of approximately 10^5 CFU per spot. Plates containing no antibiotics were inoculated before and after each antibiotic-containing series of plates were inoculated. Plates were incubated in an anaerobic chamber at 36°C for 48 h and then the growth on the plates was interpreted. The MIC was defined as the lowest concentration of antibiotic that yielded no growth, a fine haze, multiple tiny colonies, or a few discrete colonies (10). In the case of persistent light growth, the MIC was read at the concentration at which there was a marked change compared with the control growth.

TABLE 1. Biochemical test reactions and their abbreviations for the Rapid ANA II, AnIDent, and ATB 32A anaerobe identification test systems

Substrate	Abbreviation for reaction		
	Rapid ANA II	AnIDent	ATB 32A
Urease	URE		URE
Arginine dihydrolase		ARG	ADH
Glutamic acid decarboxylase			GDC
Indoxyl-acetate		INA	
Indole	IND	IND	IND
Nitrate reduction			NIT
Mannose fermentation			MNE
Raffinose fermentation			RAF
Nitrophenyls			
β,D-Disaccharide	BLTS		
α,L-Arabinoside	α-ARA		α-ARA
α,L-Arabinofuranoside		ARB	
α,D-Galactoside	α-GAL	α-GAL	α-GAL
β,D-Galactoside	ONPG	NPG	β-GAL
β-Galactoside-6-phosphate			β-GP
α,D-Glucoside	α-GLU	ADG	α-GLU
β,D-Glucoside	β-GLU	BDG	β-GLU
β,D-Glucuronidase			β-GUR
β,*N*-Acetyl-glucosaminide	NAG	NGS	β-NAG
α,L-Fucoside	α-FUC	FUC	α-FUC
Phosphate	PO_4	PHS	
Alkaline phosphatase			PAL
Arylamides and napthylamides			
Arginine	ARG	ARL	ARG A
Proline	PRO	PRO	PRO A
Leucyl-glycine	LGY		LG A
Leucyl-4-methoxy		LEU	LEU A
Glycine	GLY	GLY	GLY A
Phenylalanine	PAL	PHA	PHE A
Serine	SER		SER A
Pyrrolidonyl	PYR	PYR	
Pyroglutamic acid			PYR A
Tyrosine		TYR	TYR A
Alanine		ALA	ALA A
Histidine		HIS	HIS A
Glutamyl glutamic acid			GG A

RESULTS

Identification. The interpretation of the profile numbers generated by positive reactions with each of the anaerobe identification systems is presented in Table 2. The RapID ANA II kits identified 100% of *C. ramosum* isolates and 91% of *C. perfringens* isolates to the correct genus and species levels. However, only 24% of the *C. innocuum* isolates and 50% of the *C. clostridioforme* isolates were accurately identified to the species level. Seventy-six percent of the *C. innocuum* isolates and 40% of the *C. clostridioforme* isolates were identified to the genus level but with a low level of selectivity for species identification. According to the chart provided by the manufacturer of the RapID ANA II kits (Table 3), the arginine reaction is expected to be positive for 90% of *C. innocuum* strains; 19% of our strains were positive. Phenylalanine aminopeptidase and pyrrolidonyl amino peptidase should be positive for 80 and 76% of strains, respectively, and thus are somewhat less helpful. Our *C. innocuum* isolates were 85 and 29% positive for these two reactions, respectively. Of the *C. clostridioforme*

TABLE 2. Identification of clinical *Clostridium* isolates by anaerobe identification kits

Kit and identification	% Isolates			
	C. perfingens (n = 11)[a]	*C. ramosum* (n = 20)	*C. innocuum* (n = 21)	*C. clostridioforme* (n = 20)
RapID ANA II				
Correct genus and species	91	100	24	50
Correct genus, low level of selectivity for species	0	0	76	40
Incorrect genus or species	0	0	0	0
No identification	9	0	0	10
AnIDent				
Correct genus and species	36	60	28	90
Correct genus, low level of selectivity for species	9	40	62	5
Incorrect genus or species	0	0	5	0
No identification	55	0	5	5
ATB 32A				
Correct genus and species	46	70	0	40
Correct genus, low level of selectivity for species	9	15	52	25
Incorrect genus or species	0	0	0	0
No identification	45	15	48	35

[a] n is number of isolates tested.

strains that we evaluated, 90% were positive for α,D-galactosidase, one of the major reactions used in identifying this species (Table 3). Although 90% of the strains were expected to be positive for the α,L-arabinosidase reaction and 93% were expected to be positive for the leucyl-glycine peptidase reaction, only 40 and 40% of the isolates that we tested were positive for these reactions, respectively.

The AnIDent system identified 90% of the *C. clostridioforme* isolates, 60% of the *C. ramosum* isolates, 28% of the *C. innocuum* isolates, and 36% of the *C. perfringens* isolates to the species level. Although 40% of the *C. ramosum* isolates and 62% of the *C. innocuum* isolates could be identified to the genus level with a low level of selectivity for species identification, 55% of the *C. perfringens* isolates could not be identified even to the genus level by their profile numbers. Extremely weak glycosidase reactions were observed with the AnIDent kits. When very weak reactivity was scored as positive, many of the strains could be correctly identified. With the AnIDent system, in the case of *C. ramosum*, the α,D-glucosidase reaction was consistently weak, yet it is expected to be positive for 99% of *C. ramosum* isolates according to the manufacturer's percent chart. Seventy-six percent of the *C. innocuum* isolates were negative for the pyrrolidonyl amino peptidase reaction, which resulted in a choice overlap with other *Clostridium* species, thus accounting for the 56% of isolates identified to the correct genus level but with a low level of selectivity for the species level. *Eubacterium limosum* was listed as the first choice for 13% of the RIC isolates because the more infrequent reactions (phosphatase and β,D-glucosidase) were the only ones that scored positive.

The ATB 32A system accurately identified 70% of the *C. ramosum* isolates, 46% of the *C. perfringens* isolates, and 40% of the *C. clostridioforme* isolates to the species level. None of the *C. innocuum* isolates were identified to the correct species level; 52% of the isolates could be identified to the correct genus level with a low level of selectivity for species identification. The ATB 32A codebook had no identification numbers for *C. ramosum* isolates positive for raffinose fermentation (25% expected positive) in addition to the major reactions β,D-galactosidase, α,D-glucosidase, and β,*N*-acetylglucosaminidase. For the *C. innocuum* isolates, when the two major reactions, mannose fermentation and the pyroglutamic acid aryl amidase reaction, were positive, the code number matched a low-discrimination identification of *E. limosum*, *C. innocuum*, and *Fusobacterium varium*; these results were included in the low level of selectivity for species category. This accounted for 48% of the *C. innocuum* isolates tested. For *C. clostridioforme*, the only reactions expected to be positive greater than 80% of the time were those for α,D-galactosidase and β,D-galactosidase. However, when these two reactions were the only positive reactions, the ATB 32A codebook listed no profile number. Twenty percent of the *C. clostridioforme* strains were incorrectly identified as *C. fallax* because the two major reactions, α,D-galactosidase and β,D-galactosidase, as well as the β,D-glucuronidase reaction (22% expected positive isolates) scored positive. Five of the 11 strains of *C. perfringens* (45%) were nitrate positive in addition to being positive for the major reactions, α- and β,D-galactosidase reaction, β,*N*-acetylglucosaminidase reaction, mannose and raffinose fermentation, and pyroglutamic acid aryl amidase reaction. Although 24% of the *C. perfringens* isolates are expected to be nitrate positive, there was no corresponding profile number in the ATB 32A codebook for any profile number that included a positive nitrate reaction. One isolate was positive for alkaline phosphatase, which should be positive for 95% of the *C. perfringens* isolates according to the manufacturer's percent chart. When this reaction was positive in addition to the previously stated major reactions, the isolate was identified as *C. perfringens.*

In addition to BBA, brain heart infusion and Columbia agars were evaluated as the basal media for culturing selected isolates. When brain heart infusion agar was used, the reactions obtained were comparable to those obtained with BBA for all three identification systems. The results obtained with the RapID ANA II kits were independent of the agar used for each of the clostridial isolates tested. Likewise, the results obtained with the ATB 32A kits were essentially the same and independent of the type of agar used. However, some of the AnIDent reactions varied when the culture medium was Columbia agar rather than the standard BBA. Eight of nine (89%) *C. ramosum* isolates tested that were negative for β,D-glucosidase produced positive β,D-glucosidase reactions when they were cultured on Columbia agar. Seven of 10 (70%) *C.*

TABLE 3. Comparison of reactions obtained with *C. perfringens*, *C. ramosum*, *C. innocuum*, and *C. clostridioforme* in the RapID ANA II, AnIDent, and ATB 32A with reactions reported in the manufacturers' percent charts

Kit and test	Strains yielding positive reactions							
	C. perfringens		*C. ramosum*		*C. innocuum*		*C. clostridioforme*	
	Percent chart	Present study	Percent chart	Present study	Percent chart	Present study	Percent chart	Present study
RapID ANA II								
Urease	0	0	0	0	0	0	0	0
Glycosidases								
β,D-Disaccharidase	39	0	90	15	5	0	84	0
α,L-Arabinosidase	69	9	0	0	0	0	90	40
β,D-Galactosidase	98	100	84	0	0	0	84	50
α,D-Glucosidase	76	82	99	100	20	0	85	55
β,D-Glucosidase	36	0	99	100	36	0	87	20
α,D-Galactosidase	96	100	76	55	5	0	98	90
α,L-Fucosidase	18	36	0	0	0	0	0	0
β,*N*-Acetyl-glucosaminidase	98	100	99	100	18	0	81	25
Alkaline phosphatase	81	91	0	0	5	0	2	0
Aminopeptidases								
Leucyl-glycine	2	0	29	5	2	0	93	40
Glycine	26	0	2	0	11	0	9	0
Proline	4	0	0	0	0	0	1	0
Phenylalanine	80	100	0	0	76	29	13	0
Arginine	92	91	33	0	90	19	47	15
Serine	43	91	0	0	9	5	2	0
Pyrrolidonyl	96	100	0	0	80	85	7	35
Indole	0	0	0	0	0	0	6	5
AnIDent								
Indole	0	0	0	0	0	0	4	0
Glycosidases								
β,*N*-Acetyl-glucosaminidase	100	82	100	80	24	14	56	20
α,D-Glucosidase	95	100	99	70	2	5	56	50
α,L-Arabinofuranosidase	0	0	0	0	0	0	48	25
β,D-Glucosidase	52	9	100	75	53	9	71	35
α,L-Fucosidase	19	18	0	0	0	5	10	5
Alkaline phosphatase	96	82	0	0	7	19	0	0
α,D-Galactosidase	97	100	82	10	0	0	98	80
β,D-Galactosidase	99	63	97	15	0	5	89	50
Indoxyl-acetate	61	72	37	15	15	5	87	90
Arginine dihydrolase	35	0	0	0	0	0	0	0
Aminopeptidases								
Leucyl-4-methoxy	3	0	0	0	0	5	0	0
Proline	1	0	0	0	0	0	0	0
Tyrosine	0	0	0	0	0	0	0	0
Arginine	89	9	3	0	7	5	31	5
Alanine	0	0	0	0	0	5	0	0
Histidine	0	0	0	0	0	5	0	0
Phenylalanine	0	0	0	0	0	0	0	0
Glycine	0	0	0	0	0	0	0	0
Pyrrolidonyl	99	82	0	0	83	24	35	30
Catalase	0	0	0	0	0	0	4	0
ATB 32A								
Urease	1	0	0	0	0	0	0	0
Arginine dihydrolase	62	55	0	0	0	0	0	0

Continued on following page

TABLE 3—*Continued*

Kit and test	Strains yielding positive reactions							
	C. perfringens		*C. ramosum*		*C. innocuum*		*C. clostridioforme*	
	Percent chart	Present study	Percent chart	Present study	Percent chart	Present study	Percent chart	Present study
α,D-Galactosidase	95	100	25	5	0	0	89	85
β,D-Galactosidase	100	100	100	90	0	0	100	100
β-Galactoside-6-phosphate	14	9	80	0	10	0	6	0
α,D-Glucosidase	75	55	80	80	0	0	63	55
β,D-Glucosidase	57	9	80	70	90	5	72	50
α,L-Arabinosidase	0	0	0	0	0	0	54	40
β,D-Glucuronidase	52	63	0	0	0	0	22	50
β,*N*-Acetyl-glucosaminidase	95	100	100	85	0	0	54	50
Mannose fermentation	97	100	25	15	99	43	72	15
Raffinose fermentation	95	91	25	30	0	0	45	15
Nitrate reduction	24	45	0	0	0	0	0	0
Indole	1	0	0	0	0	0	6	5
Alkaline phosphatase	95	9	0	0	0	14	1	0
Arylamidases								
Arginine	10	0	0	0	0	0	0	0
Proline	0	0	0	0	0	0	0	0
Leucyl glycine	0	0	50	5	0	0	54	40
Phenylalanine	0	0	0	0	0	0	0	0
Leucine	10	0	0	0	0	0	0	0
Pyroglutamic acid	98	91	10	0	80	48	45	35
Tyrosine	5	0	0	0	0	0	0	0
Alanine	5	0	0	0	0	0	0	0
Glycine	5	0	0	0	0	0	0	0
Histidine	0	0	0	0	0	0	0	0
Glutamyl glutamic acid	0	0	0	0	0	0	0	0
Serine	0	0	0	0	0	0	0	0
Glutamic acid decarboxylase	48	82	0	10	0	0	0	0
α-Fucosidase	24	36	0	0	0	0	0	0

innocuum isolates tested that were negative for arginine aminopeptidase were positive when they were cultured on Columbia agar, and 3 of 10 (30%) *C. innocuum* isolates tested that were positive for phosphatase on BBA were negative on Columbia agar.

Reactions in PRAS biochemicals confirmed the saccharolytic activities of these species. The results of these biochemical fermentations, the end products of glucose metabolism, and other reactions are provided in Table 4.

Antimicrobial susceptibility testing. Each isolate used in the present study was tested for its susceptibility to 11 antimicrobial agents by the agar dilution method. The results of those tests are presented in Table 5.

All *C. perfringens* isolates were susceptible to all agents tested. In contrast, the RIC group of isolates showed resistance to certain antimicrobial agents. Some strains of *C. ramosum* were resistant to penicillin (20%), clindamycin (5%), and cephalosporins (20%). All *C. innocuum* isolates were resistant to the cephalosporins; many of these isolates were only moderately susceptible to penicillin (MIC at which 90% of isolates are inhibited, 8 μg/ml) and vancomycin (MIC at which 90% of isolates are inhibited, 4 μg/ml). Ninety percent of *C. clostridioforme* isolates were resistant to penicillin (MIC, >4 μg/ml); however, only one isolate produced β-lactamase, as detected by the cefinase test.

DISCUSSION

There is a need for the accurate identification of *Clostridium* isolates without the use of gas-liquid chromatography and many additional tests. Earlier studies on the identification of *Clostridium* species with rapid kits have included small num-

TABLE 4. PRAS biochemical fermentation and other reactions for *C. ramosum*, *C. innocuum*, and *C. clostridioforme*

Biochemical	Reaction[a]		
	C. ramosum	*C. innocuum*	*C. clostridioforme*
Arabinose	−	−	w
Cellobiose	+ (a)	+ (a)	+ (a)
Esculin	+ (a)	w	−
Hydrolysis	+	+	+
Glucose	+ (a)	+ (a)	+ (a)
Lactose	+ (a)	−	+ (a)
Maltose	+ (a)	−	+ (a)
Mannitol	+ (a)	−	−
Mannose	+ (a)	+ (a)	+ (a)
Raffinose	+ (a)	−	+ (a)
Rhamnose	−	−	+ (a)
Salicin	+ (a)	+ (a)	+ (a)
Sucrose	+ (a)	w	+ (a)
Trehalose	+ (a)	+ (a)	−
Xylose	−	−	+ (a)
Gelatin	−	−	−
Indole	−	−	−
Nitrate	−	−	−
Lecithinase	−	−	−
End products from glucose metabolism	Acetate (lactate)	Acetate, butyrate, lactate	Acetate

[a] −, negative reaction; +, positive reaction; (a), acid, pH ≤5,5; w, weak reaction.

bers of isolates of individual species (1, 3, 4, 7–9). In our study, the three kits, RapID ANA II, AnIDent, and ATB 32A, were not consistently reliable as the sole method for the identification of *C. ramosum*, *C. innocuum*, and *C. clostridioforme* isolates. Use of the ATB 32A system resulted in the greatest percentages of no identification for the RIC group. We found that reliance on variable reactions (positive for 20 to 80% of isolates) is not helpful for generating profile numbers. Nevertheless, these reactions can be helpful in identifying some of the isolates with atypical reaction profiles. When organism identification with low confidence values does occur, physical appearance on Gram stain and supplemental tests, as recommended in the manufacturers' codebooks, can distinguish among the possible identification choices. Since the codebooks cannot list the profile numbers for all of the possibilities, a number of our isolates could not be identified by the manufacturers' schemata.

Although these systems have written descriptions of the colors of positive and negative tests, interpretation of the results of these tests is not always clear (8). In each of the three identification systems that we tested, some of the results of the reactions were difficult to interpret. Whereas the overall agreement between the expected reactions and our results of individual reactions was adequate and false-positive reactions were infrequent, some of the glycosidase tests produced very weak reactions that, according to the instructions, should be interpreted as negative. However, in order to correctly identify these isolates, weakly positive reactions had to be considered positive in order to generate a code number that matched. Although in some instances the profile numbers generated excluded an accurate identification with the manufacturer's codebook, a comparison with the percent chart would have identified the isolate. Other investigators have reported similar difficulties in interpreting reactions for these isolates (7, 9). For the RIC group of isolates, correct identification to the species level was not observed consistently with any single kit tested.

TABLE 5. Susceptibilities of *C. perfringens*, *C. ramosum*, *C. innocuum*, *C. clostridioforme*, and American Type Culture Collection control strains to 11 antimicrobial agents

Organism and antimicrobial agent	MIC (μg/ml)[a]			MIC range (μg/ml) for control strain
	Range	50%	90%	
C. perfringens (ATCC 13147)[b] (11 strains)				
Penicillin G	0.25–2	1	2	0.5–1
Metronidazole	0.5–2	1	2	0.25–0.5
Clindamycin	0.06–2	0.25	2	0.06–0.125
Cefoxitin	0.5–2	2	2	1–2
Cefotetan	0.06–1	0.125	1	0.125–0.25
Imipenem	0.125–2	0.25	0.5	0.125–0.125
Meropenem	≤0.015–1	0.03	1	0.03–0.06
Amoxicillin-clavulanate[c]	≤0.03–0.25	0.03	0.25	0.06–0.25
Ampicillin-sulbactam[c]	≤0.03–0.5	0.06	0.25	0.06–0.125
Piperacillin-tazobactam[d]	0.03–4	0.125	1	0.06–0.125
Vancomycin	0.125–0.25	0.25	0.25	0.25–0.25
C. ramosum (ATCC 25582) (20 strains)				
Penicillin G	0.125–16	0.5	8	0.5–2
Metronidazole	0.06–4	0.5	1	0.5–1
Clindamycin	0.25–8	2	4	4–8
Cefoxitin	0.5–64	4	64	4–8
Cefotetan	0.125–128	1	64	1–4
Imipenem	0.25–1	0.5	1	0.25–1
Meropenem	0.125–4	2	4	1–2
Amoxicillin-clavulanate	≤0.03–0.5	0.125	0.25	0.06–0.25
Ampicillin-sulbactam	0.06–1	0.25	1	0.06–0.5
Piperacillin-tazobactam	0.06–2	0.06	1	0.06–0.25
Vancomycin	0.25–2	2	2	2–2
C. innocuum (ATCC 14501) (21 strains)				
Penicillin G	2–16	4	8	2–8
Metronidazole	0.5–1	1	1	0.5–1
Clindamycin	0.125–>32	1	1	0.25–0.25
Cefoxitin	64–128	128	128	64–128
Cefotetan	128–>128	128	>128	>128–>128
Imipenem	1–8	4	4	2–4
Meropenem	0.5–2	2	2	1–2
Amoxicillin-clavulanate	0.125–0.5	0.5	0.5	0.125–0.5
Ampicillin-sulbactam	0.25–1	0.25	0.5	0.25–0.5
Piperacillin-tazobactam	1–4	1	2	1–1
Vancomycin	2–8	4	8	4–4
C. clostridioforme (ATCC 25537) (20 strains)				
Penicillin G	2–>128	8	32	4–8
Metronidazole	≤0.03–0.5	0.06	0.125	≤0.03–0.03
Clindamycin	≤0.015–4	0.03	1	0.125–0.25
Cefoxitin	2–32	4	32	8–16
Cefotetan	1–8	2	8	2–4
Imipenem	0.125–4	2	4	1–4
Meropenem	0.125–4	2	2	0.25–1
Amoxicillin-clavulanate	0.06–4	0.25	0.5	0.25–0.5
Ampicillin-sulbactam	1–16	2	2	0.5–2
Piperacillin-tazobactam	1–128	8	16	1–4
Vancomycin	0.125–1	0.125	0.25	0.25–0.25

[a] 50% and 90%, MICs at which 50 and 90% are inhibited, respectively.
[b] The American Type Culture Collection (ATCC) strains given in parentheses are the control strains.
[c] Tested at a ratio of 2:1.
[d] Tazobactam was held constant at 4 μg/ml.

In the present study, we tested additional basal media to determine if culture conditions affected the reactivities of the isolates. Some differences in reactivities were noted depending on the system and the medium used. Although the use of Columbia agar improved the ability of some isolates to produce the expected reactions with the AnIDent kit, our data in Tables 2 and 3 were based on the results obtained with isolates grown on brucella agar.

PRAS biochemical fermentation tests, end product analysis by gas-liquid chromatography, and other reactions were able to confirm the identification of the strains. In addition, primary characteristics provide clues that help to distinguish the isolates from other genera or species (13). *C. ramosum* forms low convex, gray, opaque colonies that are 1 to 2 mm in diameter with an entire edge. Growth of *C. ramosum* on BBA turns the plate brown. Cells are Gram variable with infrequent small, terminal spores, and they often form chains when they are grown in broth media. *C. innocuum* forms fairly large, gray-white opaque colonies that are 3 to 5 mm in diameter with a slightly spreading edge. Colonies on BBA fluoresce chartreuse under long-wave UV light. Cells stain gram positive and produce infrequent spores that are terminal and that distend the cell. *C. clostridioforme* forms low convex, gray-white colonies that are 2 to 3 mm in diameter with a slightly irregular edge. Colonies on BBA produce hydrogen peroxide when they are exposed to air, which causes greening of the blood. Cells typically stain gram negative and are seed shaped with tapered ends. Spores are rarely seen, but when they are present, they are subterminal and distend the cell. In spite of the distinctly gram-negative appearance of the cells, cultures are susceptible to the 5-μg vancomycin disk, which helps to differentiate them from fusobacteria, which are resistant to vancomycin.

Previous studies testing the antimicrobial susceptibilities of *Clostridium* species have reported *C. perfringens* as a separate species, but they have combined the other species as one group of uniform organisms, even though the individual species have disparate susceptibility patterns (2, 12, 15). Whereas the *C. perfringens* isolates were uniformly susceptible to all of the antibiotics tested, the RIC group of isolates showed variations in their susceptibilities. This is often not appreciated when a varied mixture of clostridial species is tested and MICs are reported for an artificially lumped group. Thus, in cases of serious anaerobic infections, not only should the clinical laboratory be able to accurately identify *Clostridium* isolates to the species level but it is also important that the laboratory perform susceptibility tests on individual isolates from patients.

In conclusion, none of the three rapid identification kits tested in the present study can be used as the sole method for the identification of the *Clostridium* species tested. All three systems can be supplemented with additional tests for the complete identification of selected organisms. Improvements to the manufacturers' database, i.e., the inclusion of code numbers corresponding to reactions of lower expected frequency, would increase the likelihood that some of these isolates would be correctly identified. In situations in which a low level of selectivity of the test for several species is associated with a code number, familiarity with the Gram stain results and unusual cell and colonial morphologies of these species can suggest a RIC group of *Clostridium* species. PRAS biochemical fermentation tests and other reactions can also be used to differentiate between overlapping choices. In combination, the Gram stain, rapid identification kits, and additional tests can provide an accurate means for identifying the *Clostridium* RIC group of organisms.

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