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Clostridium aldenense sp. nov. and *Clostridium citroniae* sp. nov. Isolated from Human Clinical Infections

Yumi A. Warren,[1] Kerin L. Tyrrell,[1] Diane M. Citron,[1]* and Ellie J. C. Goldstein[1,2]

R. M. Alden Research Laboratory, Santa Monica, California 90404,[1] and UCLA School of Medicine, Los Angeles, California 90073[2]

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One hundred eight isolates were previously identified in our laboratory as *Clostridium clostridioforme* by colonial and cellular morphology, as well as biochemical tests. Recent studies have indicated that there are actually three different species in this *C. clostridioforme* group: *C. hathewayi*, *C. bolteae*, and *C. clostridioforme*. Our isolates were reexamined using biochemical and enzymatic tests and molecular methods. Forty-six isolates were reidentified as *C. hathewayi*, 34 as *C. bolteae*, five as *C. clostridioforme*, and one as *C. symbiosum*. Twenty-two strains were identified only to the genus level by 16S rRNA gene sequencing, and although they are microscopically and morphologically indistinguishable from the above-mentioned three species, they are phenotypically different and only 96 to 98% similar by gene sequencing. Twenty of these 22 strains were indole positive and formed two novel species. We propose *Clostridium aldenense* sp. nov. and *Clostridium citroniae* sp. nov. as names for these new species. They are differentiated from each other by results for raffinose, rhamnose, α-galactosidase, and β-galactosidase: positive, negative, positive, and positive, respectively, for the former species and negative, positive, negative, and negative, respectively, for the latter species. The type strain of *C. aldenense* is RMA 9741 (ATCC BAA-1318; CCUG 52204), and the type strain of *C. citroniae* is RMA 16102 (ATCC BAA-1317; CCUG 52203).

Clostridium species are endospore-forming, obligately anaerobic, gram-positive bacilli. By this simple definition, the genus includes species with diverse morphological, phenotypic, and biochemical properties and is the largest anaerobic genus (7, 8, 21, 31, 33, 37). These species are found widely in the environment, form a part of the normal enteric microflora of humans and animals, and are capable of causing endogenous and exogenous infections (1, 6, 8, 12, 17, 32). In fact, *Clostridium* species are one of the predominant anaerobes in the human intestinal tract (34, 35) and are a major cause of endogenous infection.

Until recently, identification of *Clostridium* organisms has been accomplished by biochemical tests and gas-liquid chromatography. While the conventional macrotube biochemical tests have been the most accurate methods of identification (20), they are expensive and time-consuming (5, 17, 35). Although the rapid methods that measure preformed enzymes are convenient, their accuracy has not been adequate for identifying clostridia (1, 5, 9, 20, 27). Therefore, basic characteristics, such as Gram stain and colony morphology, continue to play an important role in their identification (1, 2, 20). However, this approach also has limitations because some *Clostridium* strains stain gram negative and others do not consistently exhibit spores. For this reason, some clostridia, such as *Clostridium symbiosum* and *C. clostridioforme*, were previously included within gram-negative genera (9, 15). More-advanced techniques, such as 16S rRNA gene sequencing, have made identification more accurate and rearrangement by phylogenetic relatedness possible within the genus, and consequently new genera have been defined (7, 35).

C. clostridioforme is one such example, and two new species have already been described, *C. hathewayi* (32) and *C. bolteae* (28). The three species are morphologically and microscopically indistinguishable from each other, appearing as cigar-shaped, gram-negative bacilli, often connected end to end, and sometimes mistaken for fusobacteria; however, susceptibility to the 5-μg vancomycin special potency disc distinguishes them from fusobacteria (1, 15). Spores are rarely seen, but when present, they are oval and subterminal.

Identification of *C. clostridioforme* is important for several reasons. Susceptibility testing is generally not done in clinical laboratories, although resistance to antibiotics by *C. clostridioforme* has been noted, and some strains produce β-lactamase (2, 6, 13, 24, 25). In addition, *C. clostridioforme* strains produce a higher metabolic enzyme activity among the intestinal microflora, which leads to the generation of toxic or carcinogenic metabolites (22, 28). Although most *Clostridium* spp. are not generally considered to be invasive, *C. clostridioforme* organisms have been isolated from osteomyelitis (30), blood (8, 19), liver abscess (8), subgingival area (38), and diabetic foot infections (D. M. Citron and E. J. C. Goldstein, Abstr. 43rd Annu. Meet. Infect. Dis. Soc. Am., abstr. 316, 2005).

Since 1984, we have isolated and identified 149 strains of *C. clostridioforme* by using standard methods (14, 16). Finegold et al. (9) reported the biochemical reactions that distinguished *C. hathewayi*, *C. bolteae*, and *C. clostridioforme*. Based on their results, we reexamined 108 isolates of our *C. clostridioforme* group strains recovered since 1988 to reidentify them according to the newly described criteria. As a result, 46 isolates were reidentified as *C. hathewayi*, 34 as *C. bolteae*, five as *C. clostridioforme*, and one as *C. symbiosum*. The remaining 22 isolates did not match the above-mentioned species biochemi-

* Corresponding author. Mailing address: R. M. Alden Research Laboratory, 2001 Santa Monica Blvd., Suite 685W, Santa Monica, CA 90404. Phone: (310) 453-7820. Fax: (310) 453-7670. E-mail: rmarl@verizon.net.

TABLE 1. Phenotypic differentiation for five species in the *C. clostridioforme* group and three reference strains

PRAS biochemical[a]	Result for species or strain (no. of strains tested)[e]									
	C. aldenense sp. nov. (n = 13)	*C. aldenense* type strain (n = 1)	*C. citroniae* sp. nov. (n = 7)	*C. citroniae* type strain (n = 1)	*C. bolteae* (n = 33)	*C. bolteae* ATCC BAA-613^T (n = 1)	*C. clostridioforme* (n = 4)	*C. clostridioforme* ATCC 25537^T (n = 1)	*C. hathewayi* (n = 33)	*C. hathewayi* CCUG 43506^T (n = 1)
Lactose	77	+	1	−	17	−	**100**	+	**100**	+
Melezitose	**0**	−	**0**	−	83	+	**0**	−	98	+
Raffinose	**100**	+	0	−	70	+	100	+	96	+
Rhamnose	0	−	**100**	+	71	+	100	+	69	+
Salicin	8	W	0	−	16	−	100	+	97	+
Indole[b]	**100**	+	**100**	+	0	−	0	−	0	−
β-Gal[c]	100	+	0	−	100	−	50	+	100	+
ONPG[d]	23	−	29	−	**0**	−	**100**	+	50	+
α-Gal[c]	100	+	0	−	100	+	50	+	100	+
NAG[c]	0	−	0	−	0	−	0	−	**100**	+
ArgA[c]	0	−	0	−	0	−	0	−	0	−
PAL[c]	92	+	100	+	0	−	0	+	0	−
aARA[c]	92	+	0	−	0	−	0	−	100	+

[a] β-Gal, β-galactosidase; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; α-Gal, α-galactosidase; ArgA, L-arginine-β-napthylamide; PAL, 2-napthyl-phosphate; aARA, 4-nitrophenyl-αL-arabinofuropyranoside.
[b] Tested by spot indole test.
[c] Tested by Rapid ID 32A.
[d] Tested by RapID ANA II.
[e] Numbers represent percentages of positive results. Numbers in boldface type indicate key reactions. +, positive reaction to biochemical; −, negative reaction; W, weak reaction. Not all strains were tested using Rapid ID 32A.

cally, and identification to the genus level was accomplished by 16S rRNA gene sequencing. Among those 22 *Clostridium* species, 20 strains were different from all of the other species by being indole positive and by other key biochemical reactions, suggesting that they are novel species. We propose these new strains as *Clostridium aldenense* sp. nov. and *Clostridium citroniae* sp. nov.

MATERIALS AND METHODS

Clinical isolates and phenotypic identification. All 108 isolates were previously frozen in 20% skim milk maintained at −70°C; 95 isolates were from intra-abdominal specimens, four from pelvic specimens, five from skin, three from blood, and one from an unknown source. They were subcultured at least twice on *Brucella* blood agar plates with hemin and vitamin K_1 (Hardy Diagnostics, Santa Maria, CA). The type strains of *C. clostridioforme* (ATCC 25537), *C. bolteae* (ATCC BAA-613), and *C. hathewayi* (CCUG 43506) were also included.

Prereduced anaerobically sterilized (PRAS) biochemicals (Anaerobe System, Morgan Hill, CA) included in this study were arabinose, esculin, glucose, lactose, maltose, mannitol, mannose, melezitose, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, trehalose, xylose, gelatin, and indole-nitrate. The tubes were inoculated with 0.2 ml of a 0.5 McFarland standard bacterial suspension in *Brucella* broth by use of a syringe and incubated at 37°C for 72 h. The pH of each tube was measured using a pH meter (Orion Research Incorporated, Boston, MA). A pH of 5.59 or lower was positive, 5.90 or greater was negative, and 5.60 to 5.89 was considered a weak reaction. Indole was tested in PRAS indole-nitrate tubes (Anaerobe System) and by a spot indole test (Anaerobe System). Preformed enzymes were tested using Rapid ID 32A (bioMerieux, Durham, NC) and RapID ANA II (Remel, Lenexa, KS) systems, according to the manufacturers' package inserts.

Genotypic identification. Isolates that were of questionable identification by phenotypic testing were sequenced to confirm their identifications. Cellular DNA was extracted using a DNeasy tissue kit (QIAGEN, Inc., Valencia, CA). Amplification of 16S rRNA genes used two universal primers, 8UA and 907B (positions 8 and 907, *Escherichia coli* numbering) (4, 29). PCR was performed with an initial 3 min at 95°C; followed by 30 s at 95°C, 30 s at 55°C, and 60 s at 72°C, repeated 34 times; and a final extension cycle at 72°C for 5 min. PCR products were electrophoresed in a 1% agarose gel and purified using a QIAquick gel extraction kit (QIAGEN, Inc., Valencia, CA). Purified DNA was sequenced directly (Laguna Scientific Laboratory, Laguna Beach, CA) with a Biotech Diagnostic Big Dye (Biotech Diagnostics, CA) sequencing kit on an ABI 377 sequencer (Applied Biosystems, Foster City, CA). The resulting sequences were compared with sequences in the GenBank database by using BLAST software (3, 18), and the closest relatives of the unknown isolates were determined.

For the type strains of *C. aldenense* sp. nov. and *C. citroniae* sp. nov., an additional 16S subregion was amplified using universal primers 774A and 1485B (positions 774 and 1485, *E. coli* numbering) (4, 29). Sequences for each of the overlapping subregion pairs were aligned together by using MEGA version 3.1 (18), creating ~1,400-bp sequences. A multiple alignment of these almost-full-length sequences was created using MEGA3 and aligned using the native implementation of CLUSTAL W in the alignment explorer tool of MEGA3, followed by further manual correction using MEGA3 and FinchTV (Geospiza, Inc., Seattle, WA).

Phylogenetic trees were constructed with MEGA3 by using the neighbor-

TABLE 2. Profile numbers for each species, obtained by use of Rapid ID 32A and RapID ANA II systems[a]

Species	Profile no. obtained by:							
	Rapid ID 32A				RapID ANA II			
C. hathewayi	45710000	45710040	45710400	45770440	425040	425042	425240	465040
					465240	465242	475040	475240
					625242	665242	675040	675042
					675240	675242		
C. bolteae	45000000	45000400			001000	001040	001401	001441
					001631			
C. clostridioforme	00000000	41000000	41000400		011000			
C. aldenense	41006000	41202000	41206000	41266000	001004	011004		
C. citroniae	00006000				000004	001004	011004	

[a] Higher profile numbers indicate more positive reactions.

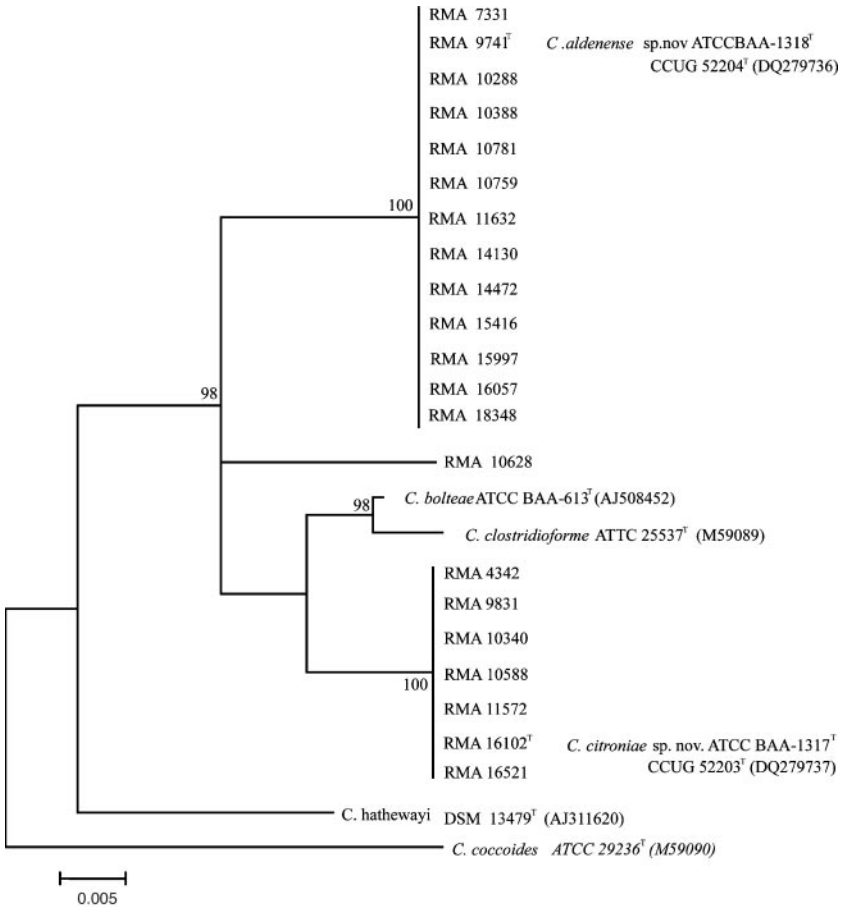

FIG. 1. Unrooted tree showing the phylogenetic positions of *Clostridium aldenense* sp. nov. and *C. citroniae* sp. nov. within the *C. clostridioforme* group rRNA cluster of organisms. The tree was constructed using the neighbor-joining method based on a comparison of approximately 700 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are given at the branching points. GenBank accession numbers are given in parentheses.

joining method (26). Reliability of the inferred trees was estimated using bootstrap analysis (500 repetitions). The resulting trees were visualized using the tree explorer tool of MEGA3.

Antimicrobial susceptibility testing. Susceptibilities of seven *C. citroniae*, 13 *C. aldenense*, 34 *C. bolteae*, five *C. clostridioforme*, and 46 *C. hathewayi* strains were determined against 11 antimicrobial agents, including ampicillin-sulbactam (Pfizer, Roerig Division, Groton, CT), piperacillin-tazobactam (Wyeth Pharmaceuticals, Pearl River, NY), ertapenem (Merck & Co., Rathway, NJ), cefoxitin (Merck & Co.), ceftriaxone (Roche Laboratories, Inc., Nutley, NJ), moxifloxacin (Bayer Corporation, Mt. Prospect, IL), levofloxacin (Johnson & Johnson, Springhouse, PA), chloramphenicol (Sigma, St. Louis, MO), penicillin (Sigma), clindamycin (Voigt Global Distributing, Kansas City, MO), and metronidazole (Searle, Inc., Skokie, IL). Antimicrobial powders were reconstituted according to the manufacturers' instructions, and serial twofold dilutions were made. Susceptibility testing was performed by the agar dilution method according to the procedure described in CLSI (formerly NCCLS) document M11-A6 (23). When the penicillin MIC result was ≥ 1 μg/ml, β-lactamase was tested using Nitrocef disks (Hardy Diagnostics, Santa Maria, CA).

Nucleotide sequence accession numbers. The almost-complete sequences of the type strains of *C. aldenense* and *C. citroniae* were deposited in GenBank under accession numbers DQ279736 and DQ279737, respectively.

RESULTS AND DISCUSSION

Phenotypic differentiation of five species in the *C. clostridioforme* group and three reference strains is shown in Table 1. The table is a summary of PRAS biochemicals and rapid systems.

Out of 108 isolates, 46 were reidentified as *C. hathewayi* by positive results for lactose, melezitose, and *N*-acetyl-β-glucosaminidase (NAG), except for one isolate that was melezitose negative, which made the overall agreement only slightly less than 100%. This isolate was confirmed as *C. hathewayi* by 16S rRNA gene sequence analysis. Five *C. clostridioforme* strains were positive for lactose and negative for melezitose and NAG as expected, and their identifications were confirmed by PCR. There were 34 *C. bolteae* strains, all solely identified by PCR. Unlike results from a previous report (9), our strains showed variable reactions in lactose, which resulted in difficulty in differentiating between *C. bolteae* and *C. clostridioforme*, although further testing determined that *o*-nitrophenyl-β-D-galactopyranoside could distinguish the species: *C. clostridioforme* showed positive results and *C. bolteae* negative.

Preformed-enzyme tests evaluated by the RapID ANA II system by Marler et al. (20) showed that the reaction patterns of *C. clostridioforme* were varied and that the reactions could change depending on the medium type and the manufacturer from which the colonies were taken. We also noticed that the RapID ANA II system produced two distinct patterns; however, our pattern differences were due to species differences only. As Table 2 shows, *C. hathewayi* produced significantly

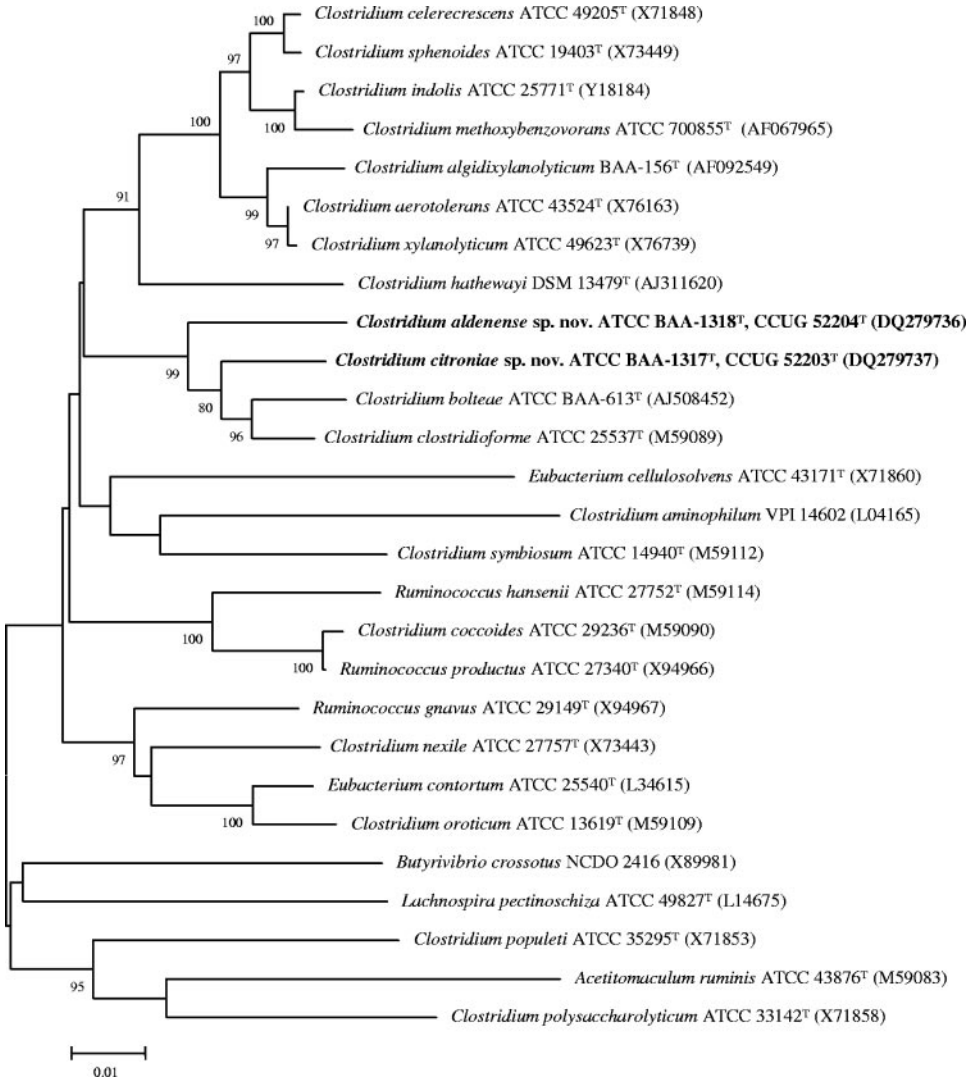

FIG. 2. Unrooted tree showing the phylogenetic positions of *Clostridium aldenense* sp. nov. and *C. citroniae* sp. nov. (in boldface type) within the *C. coccoides* rRNA cluster of organisms. The tree was constructed using the neighbor-joining method based on a comparison of approximately 1,390 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are given at the branching points. GenBank accession numbers are given in parentheses.

more positive reactions with the RapID ANA II system and somewhat more with Rapid ID 32A than *C. bolteae* and *C. clostridioforme* and there was more strain variability for many of the tests. Because *C. clostridioforme* and *C. bolteae* exist in the same genetic line but are separate from *C. hathewayi* (Fig. 1 and 2), it is understandable that they showed variations and that the preformed-enzyme systems produced two distinct profile patterns, one for *C. hathewayi* and the other for *C. bolteae*/*C. clostridioforme*, which are not in the database (9, 20).

The remaining 22 strains produced biochemical and enzymatic results that differed from those of the other three species. As the bootstrap tree shows (Fig. 1), there appeared to be two distinct groups of organisms, 13 strains in one and seven strains in the other. The 16S rRNA gene sequences of these strains did not match at the 99% level to any of the known bacterial species, while the closest known relatives were *C. bolteae* and *C. clostridioforme*. The first group showed 96 to 97% identity to *C. bolteae* and 95 to 96% identity to *C. clostridioforme*. The second group had 97 to 98% identity to both *C. bolteae* and *C. clostridioforme*. The phylogenetic tree (Fig. 2) indicated that they fit in *Clostridium* cluster XIV A as defined by Collins et al. (7). In addition, unlike the other three species, these strains were indole positive, indicating two new species in the *C. clostridioforme* group.

The MIC results are presented in Table 3. Only the ranges and the MIC_{50}s are listed for one of the two new groups and for *C. clostridioforme* due to the low numbers of isolates tested. A total of 67 strains were tested for penicillin, of which 41 strains had an MIC of $\geq$1 μg/ml and were tested for β-lactamase. Thirteen (62%) out of 21 isolates that were resistant to penicillin (MIC of >1 μg/ml) and 2 (10%) out of 20 isolates that were intermediate (MIC of 1 μg/ml) produced β-lactamase. Therefore, penicillin nonsusceptibility and β-lactamase production did not always correlate. In

TABLE 3. In vitro susceptibilities to 11 antimicrobial agents

Species (no. of isolates) and antimicrobial	MIC (μg/ml)		
	Range	50%	90%
Clostridium citroniae (*n* = 7)			
Ampicillin-sulbactam	0.125–1	0.5	NA[a]
Piperacillin-tazobactam	4–8	8	NA
Ertapenem	0.5–1	0.5	NA
Cefoxitin	4–8	4	NA
Ceftriaxone	2–4	2	NA
Moxifloxacin	8–8	8	NA
Levofloxacin	>16–>16	>16	NA
Chloramphenicol	2–4	2	NA
Clindamycin	1–4	1	NA
Metronidazole	≤0.06–≤0.06	0≤.06	NA
Penicillin	0.25–1	0.5	NA
Clostridium aldenense (*n* = 13)			
Ampicillin-sulbactam	0.5–0.5	0.5	0.5
Piperacillin-tazobactam	0.125–4	2	4
Ertapenem	0.125–0.5	0.5	0.5
Cefoxitin	2–8	4	8
Ceftriaxone	1–2	2	2
Moxifloxacin	8–16	8	16
Levofloxacin	16–>16	>16	>16
Chloramphenicol	0.5–4	2	4
Clindamycin	1–4	2	4
Metronidazole	≤0.06–0.125	≤0.06	0.125
Penicillin	0.25–0.5	0.25	0.5
Clostridium bolteae (*n* = 34)			
Ampicillin-sulbactam	0.5–16	8	16
Piperacillin-tazobactam	≤.06–>128	32	>128
Ertapenem	0.06–2	1	1
Cefoxitin	4–32	16	16
Ceftriaxone	0.5–64	4	8
Moxifloxacin	0.5–8	8	8
Levofloxacin	2–>16	>16	>16
Chloramphenicol	1–8	2	2
Clindamycin	≤0.06–4	1	2
Metronidazole	≤0.06–1	0.125	0.125
Penicillin (*n* = 17)	0.5–>4	>4	>4
Clostridium clostridioforme (*n* = 5)			
Ampicillin-sulbactam	0.5–2	1	NA
Piperacillin-tazobactam	0.25–8	0.5	NA
Ertapenem	0.5–2	0.5	NA
Cefoxitin	16–16	16	NA
Ceftriaxone	2–8	4	NA
Moxifloxacin	4–8	8	NA
Levofloxacin	16–>16	>16	NA
Chloramphenicol	2–4	2	NA
Clindamycin	≤0.06–1	0.125	NA
Metronidazole	≤0.06–≤0.06	≤0.06	NA
Penicillin	0.5–>256	0.5	NA
Clostridium hathewayi (*n* = 46)			
Ampicillin-sulbactam	0.125–8	1	1
Piperacillin-tazobactam	0.5–64	8	16
Ertapenem	0.125–4	2	4
Cefoxitin	1–8	4	4
Ceftriaxone	0.5–>128	32	64
Moxifloxacin	0.5–8	8	8
Levofloxacin	2–16	16	16
Chloramphenicol	1–32	2	4
Clindamycin	≤0.06–32	0.5	1.5
Metronidazole	≤0.06–2	0.25	0.5
Penicillin (*n* = 25)	0.5–>4	1	4

[a] NA, not applicable.

addition, the number of *C. clostridioforme* group organisms that produce β-lactamase was much higher in our study than in the study by Alexander et al. of isolates recovered between 1984 and 1993 (1). By species, 11 (65%) *C. bolteae* isolates, three (12%) *C. hathewayi* isolates, and one (20%) *C. clostridioforme* isolate produced β-lactamase in our study, while none of the new species was positive.

Many clinical laboratories do not identify anaerobes, especially to the species level (12). However, identifying some of the clinically common clostridial isolates is necessary because susceptibility is often species specific, and the importance of performing susceptibility testing on clostridial isolates other than *C. perfringens* has been shown in several studies (10, 11, 36). Our susceptibility results were different from those of the study by Finegold et al. (9) in that most strains in all five species appeared resistant to moxifloxacin by use of FDA-defined breakpoints. In general, *C. bolteae* seems more resistant than other species in this group, presenting higher MICs for penicillin, ampicillin-sulbactam, and piperacillin-tazobactam and more strains producing β-lactamase.

In this study, we identified two new species in the *C. clostridioforme* group. We propose *C. aldenense* sp. nov. and *C. citroniae* sp. nov. as names for these novel species. *C. aldenense* and *C. citroniae* are genetically closely related to *C. bolteae* and *C. clostridioforme* but are more distant from *C. hathewayi* (Fig. 2). *C. aldenense* and *C. citroniae* could be differentiated by their profile numbers obtained by Rapid ID 32A but not by RapID ANA II (Table 2). By individual testing, they could be differentiated by results for raffinose, rhamnose, α-galactosidase, and β-galactosidase, as shown in Table 1.

Prior to the identification of these newly described species, *C. clostridioforme* was one of the most frequently isolated *Clostridium* species in clinical specimens (21, 27). In a recent intra-abdominal study, we isolated 1,190 anaerobes from 401 patient specimens (unpublished data). There were 158 *Clostridium* strains, comprising 13% of the total. Thirty-seven of these (23%) were in the *C. clostridioforme* group: 19 (51%) *C. hathewayi* strains, 11 (30%) *C. bolteae* strains, two (5%) *C. clostridioforme* strains, four (11%) *C. aldenense* strains, and one (3%) *C. citroniae* strain. *C. clostridioforme* was in reality one of the least frequently encountered species among this group.

The taxonomy within the genus *Clostridium* has been in flux during the last 10 years. The *C. hathewayi* and *C. bolteae* strains in our study were recovered as early as 1998, confirming the clinical importance of *C. hathewayi* first reported by Elsayed and Zhang in 2004 (8). Interestingly, the two novel species described in this study were isolated from our clinical specimens even earlier: *C. aldenense* in 1994 and *C. citroniae* in 1988.

Description of *C. aldenense* sp. nov. *Clostridium aldenense* (Al.de.nen′se. N.L. neut. adj. *aldenense*, pertaining to R. M. Alden Research Laboratory and its first patron, Rose M. Alden Goldstein) produces colonies that are 1 to 2 mm in diameter, flat, opaque to white, and nonhemolytic on *Brucella* blood agar plates after 48 h at 37°C. The cells are 0.8 to 1.1 μm by 2 to 5 μm and stain gram negative. Spores are rarely seen, as with other species in the *C. clostridioforme* group.

Biochemically, the organisms are differentiated from existing species in the *C. clostridioforme* group by exhibiting a positive indole reaction. They produce acid from glucose, maltose, mannose, raffinose, sucrose, and xylose but not from cellobi-

ose, esculin, mannitol, melezitose, rhamnose, sorbitol, or starch. They do not hydrolyze urea, starch, or gelatin or reduce nitrate. Fermentations of arabinose, lactose, salicin, and trehalose and esculin hydrolysis are variable. Enzymatically, α-galactosidase and β-galactosidase are positive, but arginine dihydrolase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, and glycine arylamidase are negative. Variable reactions are produced by α-arabinosidase and alkaline phosphatase.

The type strain of *C. aldenense* is RMA 9741 (ATCC BAA-1318; CCUG 52204).

Description of *C. citroniae* sp. nov. *Clostridium citroniae* (Ci.tro′ni.i. N.L. gen. n. *citronii*, named after Diane M. Citron for numerous contributions to clinical anaerobic bacteriology as a clinical microbiologist and educator) produces colonies that are 1 to 2 mm in diameter, flat, opaque to white, and nonhemolytic on *Brucella* blood agar plates in 48 h at 37°C. The cells are 0.8 to 1.1 μm by 2 to 5 μm and stain gram negative. Spores are rarely seen, as with other species in this group, including *C. aldenense* sp. nov.

Similarly to *C. aldenense*, the organisms are indole positive and produce acid from glucose, maltose, mannose, rhamnose, sucrose, trehalose, and xylose but not from cellobiose, esculin, lactose, mannitol, melezitose, raffinose, salicin, sorbitol, or starch. They do not hydrolyze urea, esculin, starch, or gelatin or reduce nitrate. Fermentations of arabinose are variable. Alkaline phosphatase is positive, but arginine dihydrolase, α-galactosidase, β-galactosidase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, and glycine arylamidase are negative.

The type strain of *C. citroniae* is RMA 16102 (ATCC BAA-1317; CCUG 52203).

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