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Characterization of Ear Fluid Isolates of *Alloiococcus otitidis* from Patients with Recurrent Otitis Media

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Nineteen isolates of *Alloiococcus otitidis* from ear fluid samples collected by tympanostomy from patients at four geographic locations were identified by phenotypic characterization and genetic relatedness. Initial growth of *A. otitidis* isolates occurred after 3 days at 37°C on brain heart infusion (BHI) agar with 5% rabbit blood. Heavy growth occurred in BHI broth supplemented with 0.07% lecithin and 0.5% Tween 80 after 4 days of incubation. The isolates were gram-positive cocci that divided on an irregular plane and produced metabolic lactic acid, pyrrolidonyl arylamidase, and leucine aminopeptidase. These cocci grew sparsely in 6.5% NaCl–BHI broth, were asaccharolytic on both fermentative and oxidative bases, and were cytochrome negative by the iron-porphyrin test. The cellular fatty acid profile of *A. otitidis* was distinguished from those of related genera and characterized by major amounts (≥14%) of 16:0, 18:2, 18:1 ω9c, and 18:0 and smaller amounts of 14:0, 16:1 ω7c, 17:0, and 18:1 ω7c. Fifteen isolates demonstrated >69% relatedness by DNA-DNA hybridization. Four isolates plus the original 15 were confirmed as *A. otitidis* by dot blot hybridization with a digoxigenin-labeled nucleotide probe specific for this species. The intergenic space between the genes coding for the 16S and 23S rRNAs of alloiococci was amplified by PCR, analyzed by restriction fragment length polymorphism, and determined to consist of three different genetic types. Although β-lactamase negative, *A. otitidis* demonstrated intermediate levels of resistance to beta-lactams, including expanded-spectrum cephalosporins, and were resistant to trimethoprim-sulfamethoxazole and erythromycin.

Alloiococcus otitidis was first isolated from the ear fluid samples of children in a clinical study of chronic otitis media in Buffalo, N.Y. (10). Fifteen *A. otitidis* isolates were collected from 320 tympanocentesis fluid samples collected during effusive episodes of 200 patients with otitis media. In a study in Atlanta, Ga., of patients undergoing tympanostomy and tube placement, *A. otitidis* was isolated from 5 of 108 ear fluid samples collected from young children with chronic otitis media during 1991 and 1992 (20). The children had otalgia and middle ear effusion, with reddened, thickened, and retractive tympanic membranes. Two of the 5 Atlanta isolates and 10 of the 15 New York isolates were the only organisms found in the chronically effusive ear fluid samples, indicating a potential association between this bacterium and chronic effusive otitis. In 1992, British researchers designated this organism a new genus and species, *A. otitis*, on the basis of sequence analysis of the 16S rRNA (1). This nomenclature was revised to *A. otitidis* in keeping with the rules of the Bacteriological Code (21).

This study characterizes 19 of these isolates from four geographic locations to (i) determine an adequate growth medium for cultivation, (ii) identify physiological characteristics, (iii) confirm the identities of these isolates by using DNA-DNA homology and a dot blot probe, (iv) subtype isolates by PCR-based restriction fragment length polymorphism (RFLP), and (v) determine the MICs of nine antibiotics commonly used to treat patients with otitis media.

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MATERIALS AND METHODS

Bacterial strains. Twelve of 15 ear fluid isolates from a New York study of otitis media (10) were submitted as unidentified gram-positive cocci to the Centers for Disease Control and Prevention (CDC). These isolates are designated EF-1 to EF-12 in this study. Five isolates were from Georgia (EF-14 and EF-16 to EF-19) (20), one was from Michigan (EF-15), and one was from Brazil (EF-20). All isolates were recovered at the time of tympanostomy and tube placement. One blood isolate (B-13) which was indistinguishable from *A. otitidis* by routine diagnostic methods was included. Because no type strain had been designated at the time of DNA-DNA hybridizations, strain EF-5 was chosen for genetic comparison with the other strains in this study. Strain EF-2 was later designated as type strain NCFB 2890 by the investigators responsible for naming the genus (1). Other type strains of gram-positive cocci used in comparative studies are shown in Table 1.

Growth studies. Strains were tested for growth in aerobic and anaerobic (8) atmospheres and in the presence of 5% CO_2 at 10, 30, and 37°C. All commercial media were supplied by Difco Laboratories, Detroit, Mich., unless otherwise noted. *A. otitidis* isolates were tested for growth on the following agar-based media: Trypticase soy agar plus 5% sheep blood; brain heart infusion (BHI) agar and heart infusion (HI) agar with and without 5% rabbit blood; BHI agar with 0.07% lecithin and 0.5% Tween 80 (BHIS agar); chocolate agar plus hemin, NAD, and yeast extract; Mueller-Hinton agar with and without 5% lysed horse blood (BBL, Cockeysville, Md.); Columbia agar without antibiotics; brucella agar; and buffered charcoal yeast extract. They were tested in the following broth media: Todd-Hewitt broth with and without 0.5% yeast extract or 5% horse serum; De Man, Rogosa, and Sharp broth; fluid thioglycolate (BBL); brucella broth; BHI broth; BHI broth with 0.07% lecithin and 0.5% Tween 80 (BHIS broth); and BHI broth and HI broth with 6.5 or 10% NaCl. The broth bases tested for biochemicals were purple broth base with peptones and beef extract, phenol red HI broth, and bromcresol purple HI broth.

Biochemical reactions and detection of enzymes. Organisms were tested for the biochemical reactions recommended for the identification of gram-positive cocci or other unidentified organisms (7, 9) as well as for the presence of enzymes by using the API 20S system and the API ZYM system (Analytab Products, Division of Sherwood Medical, Plainview, N.Y.). The directions of the manufacturer were followed without modification. Inocula were grown on BHIS agar for 4 days prior to cytochrome analysis by the iron-porphyrin method, as previously described (23).

Gas chromatography for end products and cellular fatty acids. Isolates were tested for the metabolic end products of glucose as reported previously (8, 12). For cellular fatty acids (CFA), cells were saponified, and the liberated fatty acids were methylated before analysis by capillary gas-liquid chromatography. Deter-

TABLE 1. Related species for comparative studies with *A. otitidis*

Species and type strain[a]	Comparative test(s)
Aerococcus urinae 2T893 (*Aerococcus hominis* NCTC 12142)	DNA-DNA, probe, fatty acids
Aerococcus viridans ATCC 11563	DNA-DNA, probe, fatty acids
Bacillus subtilis ATCC 6051	Probe
Enterococcus casseliflavis ATCC 25788	Fatty acids
Enterococcus faecium ATCC 19434	Fatty acids
Enterococcus mundtii NCDC 2375	Fatty acids
Gemella haemolysans ATCC 10379	DNA-DNA, probe
Gemella morbillorum ATCC 82427	DNA-DNA, probe
Lactobacillus delbrueckii ATCC 12315	Fatty acids, probe
Lactobacillus lactis ATCC 19435	Fatty acids, probe
Lactobacillus raffinolactis NCDC 617	Fatty acids
Lactobacillus cremoris ATCC 19257	Fatty acids
Leuconostoc mesenteroides ATCC 8293	Fatty acids, probe
Listeria monocytogenes ATCC 15313	Fatty acids, probe
Pediococcus damnosus ATCC 29358	Fatty acids, probe
Tetragenococcus halophilus ATCC 33315	DNA-DNA, fatty acids
Streptococcus equinus ATCC 9812	Probe
Streptococcus pyogenes NCDC 2381	Probe
Streptococcus uberis ATCC 19436	Fatty acids, probe
Vagococcus fluvivalis NCDC 2497	DNA-DNA, probe

[a] ATCC, American Type Culture Collection; NCDC, National Collection of Dairy Cultures; NCTC, National Collection of Type Cultures.

minations of double-bond positions in monounsaturated acids were accompanied by gas-liquid chromatography and gas-liquid chromatography–mass spectrometry (15, 16).

DNA-DNA hybridization procedures. Fifteen ear fluid isolates, one blood isolate, and five closely related species were compared for DNA relatedness to strain EF-5. DNA was isolated and purified as previously described (11). DNA hybridizations were performed by the hydroxyapatite method at 55 and 70°C and compared with isolate EF-5, which had been labeled in vitro with [^{32}P]dCTP before relative binding ratios were determined (5).

Digoxigenin-labeled DNA probe. A 16S rRNA primer sequence (1, 2) was synthesized, purified, and labeled with digoxigenin (3, 6). DNA was isolated and purified by the universal method (11) from all isolates and 14 other type strains of gram-positive species (Table 1). They were blotted, hybridized, and detected by using the Genius 3 nucleic acid detection kit (Boehringer Mannheim, Indianapolis, Ind.) (19).

PCR amplification and restriction of amplicons. Cells were grown and DNA was isolated and purified as described earlier (11). Two oligonucleotide primers, Rpc5, located at the 3′ end of the gene coding for 16S rRNA, and R23S2693, located 440 bases from the 5′ end of the gene coding for 23S rRNA in *Escherichia coli*, were used for PCR amplification of the spacer region (6, 14, 22). Amplification was performed with a Gene Amp PCR System 9600 (Perkin-Elmer). Cycles 1 through 3 included denaturation at 94°C for 70 s, annealing for 2.5 min at 55°C, and extension for 3 min at 72°C. The profile for cycles 4 through 34 was the same except that the denaturation step was shortened to 30 s and the final extension was 10 min. Amplicons were digested with *Hin*fI, (Boehringer Mannheim), and the fragments were electrophoresed and detected as described previously (13).

Antibiotic susceptibilities. The MICs of nine antibiotics were determined by using Sensititre MIC plates (Radiometer America, Westlake, Ohio) according to National Committee for Clinical Laboratory Standards dilution methods and standards (17). The method was modified by using BHI broth containing 2 IU of thymidine phosphorylase per ml and 5% lysed horse blood as the substrate. The presence of β-lactamase was determined by using nitrocefin on microdilution plates as previously described (18).

RESULTS

Growth studies. All isolates grew heavily in BHIS broth after 4 days of incubation at 37°C. They grew sparsely in BHI broth and HI broth with or without 5% CO_2 after 3 days at 37°C. No growth occurred in any other broth medium tested after 14 days of incubation. Alloiococci grew lightly in BHI broth containing 6.5% NaCl; however, none grew in BHI broth containing 10% NaCl.

All isolates grew heavily on BHI agar with 5% rabbit blood after 3 days at 37°C and moderately on HI agar with rabbit blood. A few isolates grew on Trypticase soy agar with 5% sheep blood after 5 days of incubation. None grew on any other routine isolation agar, including chocolate agar with NAD and hemin. No growth occurred at an incubation temperature of either 10 or 45°C. The rabbit blood in the BHI agar was partially hemolyzed at 3 days and after 96 h demonstrated "rusting" of the blood cells surrounding colonies. These isolates grew under aerobic conditions but did not require CO_2.

Colonial morphology. The colonies formed on BHI agar with 5% rabbit blood were small, moist, and slightly yellow at 72 h. At ≥96 h of incubation, the colonies became dark yellow, dense, hard, involuted, and adherent to agar.

Biochemical reactions and distinguishing characteristics. The organisms grew inadequately in the biochemical reagents recommended for standard identification by the CDC Reference Laboratory (9) or for the identification of either fermentative or nonfermentative bacteria by the CDC Special Bacteriology Laboratory (7). All *A. otitidis* isolates were unidentifiable with the API 20S and API ZYM systems. However, they were pyrrolidonyl arylamidase and leucine aminopeptidase positive (Table 2).

Gas chromatographic analysis for CFA and metabolic end products. The CFA compositions of *A. otitidis* and four other related genera of gram-positive cocci are presented in Table 3. The CFA compositions of the 15 *A. otitidis* strains were essentially identical and contained large amounts (26 to 34%) of 16:0 and 18:1 ω9c, moderate amounts (14 to 15%) of 18:2 and 18:0, and smaller amounts (1 to 3%) of 14:0, 16:1 ω7c, 17:0, and 18:1 ω7c. The *A. otitidis* CFA profile was unique; these isolates, also, produced lactate as the major acid from the metabolism of glucose.

DNA-DNA homology. All 15 *A. otitidis* strains tested were at least 76% related to strain EF-5 when hybridized at 55°C; 12 were at least 85% homologous (Table 4). The one blood isolate that phenotypically resembled an alloiococcus was only 3% related, with a divergence of 13.4%, indicating that it is a different species.

Digoxigenin-labeled probe specific for *A. otitidis*. The digoxigenin-labeled probe was tested with 19 ear fluid isolates and 14 other type strains of gram-positive cocci (Table 1). All ear fluid isolates were probe positive, while all others were negative.

Amplicons of the intergenic spacer region of 16S and 23S rRNAs. Figure 1 demonstrates the RFLP of the intergenic spacer region between the genes coding for the 16S and 23S rRNAs of 15 *A. otitidis* isolates following digestion with *Hin*fI. The restriction pattern of strain EF-2 (lane 2) matched those of seven other isolates; this pattern was designated amplitype A. The remaining isolates were distinguishable as two additional RFLP types, designated amplitypes B and C.

Antibiotic MICs. All of the alloiococci were β-lactamase negative. Table 5 shows the MIC ranges and modes of nine antibiotics for *A. otitidis*. All strains were resistant to trimethoprim-sulfamethoxazole (≥4/76 μg/ml) and, except for one strain, to erythromycin (≥4.0 and 0.5 μg/ml, respectively). For three isolates, the chloramphenicol MIC was 8 μg/ml; for four isolates, it was 4 μg/ml. Assuming that the resistance of *A. otitidis* can be measured with the same interpretive values as that of *Streptococcus pneumoniae*, these isolates were either susceptible or intermediately resistant to penicillin and ampicillin (range, 0.06 to 0.5 μg/ml). This same level of relative resistance was reflected in the MICs of expanded-spectrum cephalosporins as well.

DISCUSSION

Otitis media with effusion is one of the most common infections of early childhood. Thirty percent of these cases recur

TABLE 2. Biochemical reactions and characterizations of *A. otitidis* and related gram-positive bacteria[a]

Characteristic or test[b]	Result										
	Alloiococci[c]	Aerococci	Tetra-genococci[d]	Gemellae	*Aerococcus urinae*[e]	Pediococci[f]	Lactococci	Streptococci (non-beta)[g]	Enterococci	Vagococci[h]	Leuconostocs
PYR	+	+	+	+, −	−	−	+, −	−	+	+	−
LAP	+	−	+	+, −	+	+	+	+	+	+	−
Growth (24 h)[i]	−	+	−	−	+	−, +	+, −	+, −	+	+	+
Division (irregular plane)	+	+	+	+	+	+	−	−	−, +	−	−
Fermentation or oxidation of carbohydrates[j]	NG	+	+	(+)	+	+	+	+	+	+	+
Vancomycin resistance[k]	S	S	S	S	S	R	S	S	S, R	S	R

[a] Gram-positive cocci which produce metabolic lactic acid and are cytochrome negative (iron-porphyrin and benzidine peroxide tests).
[b] PYR, pyrrolidonyl arylamidase; LAP, leucine aminopeptidase.
[c] Sparse growth in 6.5% NaCl.
[d] Halophilic.
[e] Species nova (*Aerococcus hominis* NCTC 12142).
[f] Limited species tested.
[g] Other than pyroxidal-requiring cocci.
[h] Motile.
[i] BHI agar + 5% rabbit blood, 35°C, CO_2.
[j] Results of either fermentative or oxidative bases containing carbohydrates (7). Gemellae produced indiscriminately small amounts of acids. NG, no growth in biochemical basal media. Leuconostocs produced gas from glucose in De Man, Rogosa, and Sharp broth.
[k] S and R, susceptible and resistant to vancomycin (30-μg disc), respectively (4).

and require repetitive visits to physicians with numerous regimens of antibiotics. Many of these ear fluid samples are culture negative; for example, in the Georgia study, 47 of 108 patients were negative by culture (20). It is unknown whether this is due to a true sterile effusion, previous antimicrobial therapy, or a failure to culture fastidious organisms such as *A. otitidis*. We obtained isolates from cases of otitis media with effusion from four geographic locations collected over a period of 5 years, and in 12 of these, *A. otitidis* was the only organism isolated. This suggests a possible association between *A. otitidis* and chronic otitis with effusion; Faden and Dryja (10) were the first to suggest a role for this unique organism in chronic otitis. However, as yet it has no proven pathogenic role.

We tested both agar and broth media for the growth of *A. otitidis* and observed the best growth in BHIS broth and on BHI agar containing 5% rabbit blood. Even though growth was sufficient for most laboratory studies on agar medium, BHIS broth was needed to support the growth of large quantities of alloiococci for characterization studies. It is obviously a fastidious organism with rather unique nutritional requirements. Because *A. otitidis* grew so poorly in biochemical media and because it could not be identified with the API 20S or API

TABLE 3. CFA compositions of *Alloiococcus*, *Aerococcus*, *Streptococcus*, *Enterococcus*, and *Pediococcus* isolates

Fatty acid[a]	% of total fatty acids in[b]:						
	Alloiococci (15)	*Aerococcus* group 1[c] (3)	*Aerococcus* group 2[c] (34)	Streptococci[a] (175)	*Enterococcus* group 1[c] (166)	*Enterococcus* group 2[c,d] (8)	Pediococci[c] (20)
12:0	tr	tr	tr	4	ND	tr	ND
14:0	1	4	4	8	10	12	2
15:0	tr	ND	ND	ND	ND	1	1
16:1 ω9c	tr	13	16	7	ND	ND	ND
16:1 ω7c	1	ND	ND	5	8	6	2
16:1 ω5	ND	ND	ND	2	ND	ND	ND
16:1 ω9t	ND	4	4	ND	ND	ND	5
16:0	34	22	21	30	36	30	39
17:0	1	ND	ND	ND	ND	ND	ND
18:2	15	2	2	tr	2	1	2
18:1 ω9c	26	20	27	7	1	1	1
18:1 ω7c	3	4	4	19	23	38	35
18:0	14	9	8	8	5	3	6
19:0 9-10 cyc	ND	8	ND	ND	ND	ND	4
19:0 11-12 cyc	ND	ND	ND	ND	12	ND	ND
20:1 ω9c	tr	13	13	6	ND	ND	ND

[a] The number before the colon is the number of carbon atoms; the number after the colon is the number of double bonds; ω, the double bond position from the hydrocarbon end of the chain; c, the *cis* isomer; t, the *trans* isomer; cyc, cyclopropane ring.
[b] Data are arithmetic means, and numbers in parentheses indicate the numbers of strains tested. tr, trace (0.4 to 0.8%); ND, not detected.
[c] Data published previously (4, 16).
[d] Consists of *Enterococcus casseliflavis* and *Enterococcus gallinarum.*

TABLE 4. DNA-DNA hybridizations of *A. otitidis* isolates

Isolate	% Relatedness to labeled DNA from strain EF-5[a]		
	RBR at 55°C	D	RBR at 70°C
EF-5[b]	100		100
EF-1	76	1.7	69
EF-2^T	87	2.1	77
EF-3	85	1.9	72
EF-4	90	4.3	77
EF-6	92	0.4	88
EF-8	85	3.1	77
EF-9	83	2.6	76
EF-10	100	2.8	80
EF-11	93	1.9	86
EF-12	100	2.9	81
B-13[c]	3	13.4	2
EF-14	88	2.1	75
EF-16	94	2.8	76
EF-17	92	2.3	83
EF-15	90	2.1	83
Aerococcus urinae[d] NCTC 12142^T	5		
Aerococcus viridans ATCC 11563^T	5	17.1	
Gemella haemolysans ATCC 10379^T	4		
Gemella morbillorum ATCC 82427^T	5		
Tetragenococcus halophilus ATCC 33315^T	5	13.5	

[a] RBR, relative binding ratio (percent homology); D, percent divergence of unpaired bases in related DNA sequences.
[b] Labeled with [^{32}P]dCTP.
[c] Blood isolate.
[d] *Aerococcus hominis.*

ZYM system, we analyzed CFA contents, DNA-DNA relatedness, and DNA specificity, and these analyses revealed that these ear fluid isolates belong to the same genus and species. They were further divided by PCR amplification of the intergenic spacer region. Three amplitypes, A, B, and C, were found; they were not limited to a particular geographic location but appear to be widely distributed. All three types were represented among the New York isolates, the five Georgia isolates included two types, and the single isolates from Brazil and Michigan were amplitypes A and B, respectively. Amplitype A was found most often. Because these isolates were collected over a 5-year period from different locations, it is not surprising that they do not represent a single clone. However, PCR amplification of the spacer region may allow characterizations of specific strains in epidemiologic studies.

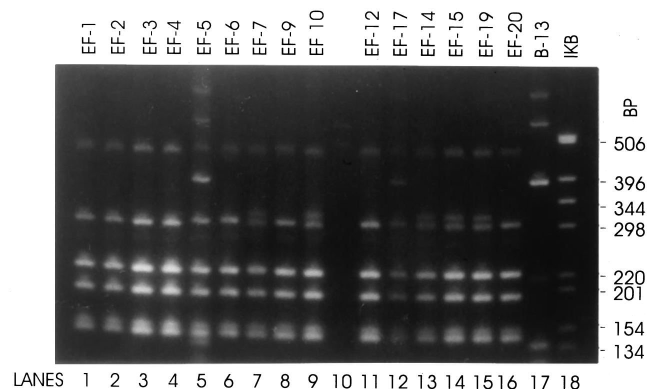

FIG. 1. *Hin*fI restriction patterns of the PCR-amplified intergenic spacer region between the genes coding for the 16S and 23S rRNAs of *A. otitidis*. Lanes 1 to 4, 6, 8, 11, and 16, amplitype A; lanes 7, 9, and 13 to 15, amplitype B; lanes 5 and 12, amplitype C; lane 17, DNA from the blood isolate; lane 10, empty; lane 18, 1-kb λ DNA ladder (1KB).

TABLE 5. Antimicrobial susceptibilities of 19 *A. otitidis* isolates

Antibiotic	MIC (mg/liter)	
	Range	Mode
Penicillin	0.06–0.12	0.06
Ampicillin	0.12–0.5	0.12
Amoxycillin-clavulanic acid	≤2/1	≤2/1
Erythromycin	0.50–16	16.0
Trimethoprim-sulfamethoxazole	≥4/76	≥4/76
Cefixime	0.5–≥1	≥1
Ceftriaxone	0.12–≥2.0	0.5
Chloramphenicol	1.0–8.0	4.0
Tetracycline	0.25–8.0	0.25

The relatedness shown in DNA-DNA hybridizations, the specificity of the probe, and the distinguishing CFA profiles indicate that *A. otitidis* is a single species and confirm the initial designation based solely on 16S rRNA gene sequencing (1) The isolation of *A. otitidis* strains which are resistant to both erythromycin and trimethoprim-sulfamethoxazole and relatively resistant to beta-lactams suggests the need to determine the significance of these cocci in chronic effusive otitis media.

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REFERENCES

1. **Aguirre, M., and M. D. Collins.** 1992. Phylogenetic analysis of *Alloiococcus otitis* gen. nov., sp. nov., an organism from human middle ear fluid. Int. J. Syst. Bacteriol. **42:**79–83.
2. **Aguirre, M., and M. D. Collins.** 1992. Development of a polymerase chain reaction-probe test for identification of *Alloiococcus otitis*. J. Clin. Microbiol. **30:**2177–2180.
3. **Becker, C. R., J. W. Efcavitch, C. R. Heiner, and N. F. Kaiser.** 1985. Use of a reverse phase column for the HPLC purification of synthetic oligonucleotides. J. Chromatogr. **326:**293–299.
4. **Bosley, G. S., P. L. Wallace, C. W. Moss, A. G. Steigerwalt, D. J. Brenner, J. M. Swenson, G. A. Hebert, and R. R. Facklam.** 1990. Phenotypic characterization, cellular fatty acid composition, and DNA relatedness of aerococci and comparison to related genera. J. Clin. Microbiol. **28:**416–421.
5. **Brenner, D. J., A. C. McWhorter, J. K. Leete Knudson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. **15:**1133–1140.
6. **Brosius, J., T. J. Dull, and H. F. Noller.** 1980. Complete nucleotide sequence of a 23S ribosomal RNA gene from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77:**201–204.
7. **Clark, W. A., D. G. Hollis, R. E. Weaver, and P. Riley.** 1984. Identification of unusual pathogenic gram negative aerobic and facultative anaerobic bacteria. U.S. Department of Health and Human Services publication. Centers for Disease Control, Atlanta.
8. **Dowell, V. R., and T. M. Hawkins.** 1974. Laboratory methods in anaerobic bacteriology. Center for Disease Control laboratory manual. U.S. Department of Health, Education, and Welfare publication no. **74:**8272. Center for Disease Control, Atlanta.
9. **Facklam, R. R., and J. A. Washington II.** 1991. *Streptococcus* and related catalase-negative gram-positive cocci, p. 238–257. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
10. **Faden, H., and D. Dryja.** 1989. Recovery of a unique bacterial organism in human middle ear fluid and its possible role in chronic otitis media. J. Clin. Microbiol. **27:**2488–2491.
11. **Graves, L. M., and B. Swaminathan.** 1993. Universal bacterial DNA isola-

tion procedure, p. 617–628. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
12. **Lombard, G. L., and V. R. Dowell, Jr.** 1982. Gas-liquid chromatography analysis of the acid products of bacteria. Centers for Disease Control, Atlanta.
13. **Matar, G. M., B. Swaminathan, S. B. Hunter, L. N. Slater, and D. F. Welch.** 1993. Polymerase chain reaction-based restriction fragment length polymorphism analysis of a fragment of the ribosomal operon from *Rochalimaea* species for subtyping. J. Clin. Microbiol. **31:**1730–1734.
14. **McClelland, M., C. Petersen, and J. Welsh.** 1992. Length polymorphisms in tRNA intergenic spacers detected by using the polymerase chain reaction can distinguish streptococcal strains and species. J. Clin. Microbiol. **30:**1499–1504.
15. **Moss, C. W., and M. A. Lambert-Fair.** 1989. Location of double bonds in monounsaturated fatty acids of *Campylobacter cryaerophila* with dimethyl disulfide derivatives and combined gas chromatography-mass spectrometry. J. Clin. Microbiol. **27:**1467–1470.
16. **Moss, C. W., P. L. Wallace, and G. S. Bosley.** 1989. Identification of monounsaturated fatty acids of *Aerococcus viridans* with dimethyl disulfide derivatives and combined gas chromatography-mass spectrometry. J. Clin. Microbiol. **26:**2387–2390.
17. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
18. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of β-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1:**283–288.
19. **Popovic, T., C. A. Bopp, Ø. Olsvik, and J. A. Kiehlbauch.** 1993. Ribotyping in molecular epidemiology, p. 573–583. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
20. **Sih, T., B. Schwartz, G. Bosley, and R. Facklam.** 1992. Chronic otitis media with effusion caused by *Alloiococcus otitis*: clinical and laboratory features, abstr. 671, p. 222. *In* Program and abstracts of the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
21. **von Graevenitz, A.** 1993. Revised nomenclature of *Alloiococcus otitis*. J. Clin. Microbiol. **31:**472.
22. **Wilson, K. H., R. B. Blitchingont, and R. C. Greene.** 1992. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. J. Clin. Microbiol. **28:**1942–1946.
23. **Wong, J. D.** 1987. Porphyrin test as an alternative to benzidine test for detecting cytochromes in catalase-negative gram-positive cocci. J. Clin. Microbiol. **25:**2006–2007.