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Two Strains of *Vibrio* Species with Unusual Biochemical Features Isolated from Ear Tracts

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A strain of *Vibrio cholerae* Heiberg type II, not agglutinable with any of the eight antisera corresponding to Heiberg's groups, and a nonmotile, methyl red-positive, encapsulated strain of *Vibrio alginolyticus* were isolated from two cases of chronic external otitis.

Vibrio species other than *Vibrio cholerae* serotype 0:1 or *V. parahaemolyticus* strains are seldom isolated from extraintestinal sites. We report here the isolation of a strain of nonagglutinable *V. cholerae* and a strain of *V. alginolyticus* with unusual biochemical features, both isolated from cases of chronic external otitis.

Case 1. A 12-year-old Moroccan girl (born in Belgium) was admitted to the hospital with a fracture of the hip bone. For several months she had suffered from untreated external otitis. A pure growth of *V. cholerae* which failed to agglutinate in cholera antiserum O, group 1, was obtained from an ear swab; the lesion responded to topical care.

Case 2. A 12-year-old Italian boy living in Belgium was seen as an outpatient in the Department of Otolaryngology for treatment of chronic external otitis. *Escherichia coli* and *Proteus mirabilis* were grown from a first sample of ear pus; 2 months later, ear draining yielded a mixed culture of *V. alginolyticus, Pseudomonas stutzeri,* and diphtheroids. The outcome of this infection is unknown since the boy was lost to follow-up.

Both strains were cultured on horse blood agar incubated at 37°C under normal conditions. Identification and antibiotic susceptibility testing were performed by conventional methods (1, 4). Since the strain of *V. alginolyticus* grew only

TABLE 1. *Characteristics of the V. cholerae and V. alginolyticus strains studied*

Test	Case 1 *V. cholerae* Heiberg type II	Case 2 *V. alginolyticus*	Test	Case 1 *V. cholerae* Heiberg type II	Case 2 *V. alginolyticus*
Oxidase	+	+	Lactose	+	−
Cytochrome oxidase	+	+	Galactose	+	+
Motility	+	−	Sucrose	+	+
Catalase	+	+	Maltose	+	+
$NO_3^- \rightarrow NO_2^-$	+	+	Raffinose	−	−
Beta-galactosidase	+	−	Arabinose	−	−
Arginine dihydrolase	−	−	Xylose	−	−
Ornithine decarboxylase	+	−	Rhamnose	−	−
Lysine decarboxylase	+	+	Mannose	−	+
Gelatinase	+	+	Melibiose	−	−
Urease	−	−	Glycerol	+	+
Indole	+	+	Mannitol	+	+
Phenylalanine deaminase	−	−	Inositol	−	−
Tryptophan deaminase	−	−	Sorbitol	−	−
Hydrogen sulfide	−	−	Dulcitol	−	−
Methyl red	−	+	Adonitol	−	−
Voges-Proskauer	+	+	Growth on anaerobic blood agar	+	+
Tetrathionate reductase	+	+			
0/129 inhibition	+	+	Growth on salmonella-shigella agar	−	−
Simmons citrate	+	+			
Malonate	−	−	Growth on MacConkey agar	+	+
Esculin	−	+			
Glucose	+	+	Growth on thiosulfate-citrate-bile-saccharose agar	+	+

in the presence of added salt, 1% NaCl was incorporated into the routine identification medium; Mueller-Hinton agar was supplemented with 3% NaCl for susceptibility testing.

Table 1 shows the biochemical reactions of both isolates. The strain isolated from case 1 was identified as *V. cholerae* belonging to group II of Heiberg (saccharose positive, arabinose negative, mannose negative) and nonagglutinable in cholera antiserum. Identification was confirmed by A. Dodin at the Pasteur Institute in Paris, who also stated that this strain did not agglutinate with any of the eight antisera corresponding to modified Heiberg groups.

The strain isolated from case 2 proved to be *V. alginolyticus* but lacked motility and was encapsulated. These unusual features were later confirmed by C. Richard at the Pasteur Institute in Paris, who noticed that this strain was the first nonmotile one out of the 130 strains he had studied. Moreover, our isolate was methyl red positive, a biochemical reaction which does not conform to the data of Feeley and Balows (1). Antibiotic susceptibilities were similar to those published previously (2, 7): both strains were resistant to penicillin G, ampicillin, carbenicillin, and vancomycin and susceptible to cephalothin, tetracycline, chloramphenicol, gentamicin, tobramycin, and co-trimoxazole. We also found them to be resistant to colistin, an observation in contradiction to that of Von Graevenitz and Carrington but which could be explained by the addition of 3% NaCl to Mueller-Hinton agar (7).

To our knowledge *V. cholerae* (all antigen groups) has never been recovered from a draining ear, and only a few publications report isolation of *V. alginolyticus* from ear tracts (3, 5–7). In most of the literature, the pathogenicity of the isolates was difficult to assess since the strains were often isolated in mixed cultures obtained from patients with known exposure to seawater. From this point of view, our cases do not differ from those published, except that exposure to a marine environment could not be established.

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