## Isolation of *Candida albicans* and Halophilic Vibrio spp. from Aquatic Birds in Connecticut and Florida<sup>†</sup>

JOHN D. BUCK

Department of Marine Sciences and Marine Sciences Institute, Marine Research Laboratory, The University of Connecticut, Noank, Connecticut 06340

Received 17 August 1989/Accepted 18 December 1989

Halophilic vibrios were recovered from feces of six types of aquatic birds (gulls, pelicans, Canada geese, swans, egrets, cormorants) from Connecticut and/or Florida shorelines. *Candida albicans* was isolated from gulls and Canada geese in Connecticut and from gulls and cormorants in Florida.

An earlier study demonstrated the occurrence of *Candida albicans* in gull feces from Connecticut and Florida (1). The research was subsequently expanded to include other species of birds and the determination of halophilic *Vibrio* spp.

Gulls are scavengers which frequent sewage treatment plants (5) and trash dumps (8). Consequently, a wide variety of human and animal pathogens have been recovered from gull feces and include, in addition to *C. albicans*, species of *Campylobacter* (7), *Listeria* (6), *Salmonella* (5), and *Yersinia* (19), as well as viruses (12). Because gulls often travel long distances (20) and other birds may eat gull feces (22), the spread of pathogens by gulls and other species over large areas seems likely.

Vibrio cholerae has been reported from geese (18), mute swans (14), white pelicans, several species of gulls and other aquatic birds (16), and unidentified coastal birds (17). Vibrio metschnikovii and V. mimicus have been found in unspecified marine birds (4), and V. parahaemolyticus occurs in unidentified aquatic birds (11).

Vibrios have emerged as serious human pathogens via raw seafood consumption and wounds received in contact with marine materials (4, 10). The ubiquity of certain birds (e.g., gulls) along coastlines and the close association of birds with human activities such as waste disposal may be significant factors in the spread of large numbers of vibrios and other pathogens to areas of importance to human health, including fishing boats, fish processing areas, and shellfish beds.

*Candida* species are also assuming increased significance as human pathogens and are now the fourth most commonly recovered blood culture isolate at some medical facilities (21). Immunosuppression is becoming a more frequent occurrence and represents a major factor in yeast and vibrio infections.

Fresh bird feces were collected by using Aerobic Transport System swabs (BBL Microbiology Systems, Cockeysville, Md.) and processed in the laboratory within a few minutes. Birds examined along beaches in southeastern Connecticut included herring gulls (*Larus argentatus*), great black-backed gulls (*L. marinus*), Canada geese (*Branta canadensis*), and mute swans (*Cygnus olor*). Florida species studied were herring gulls, laughing gulls (*L. atricilla*), ring-billed gulls (*L. delawarensis*), brown pelicans (*Peleca-*

nus occidentalis), snowy egrets (Egretta thula), and doublebreasted cormorants (*Phalacrocorax auritus*). All samples were collected in the Sarasota area from fishing piers and boat docks.

For C. albicans determination, the top portions of plates containing Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) with 150 mg of chloramphenicol per liter  $(SD^+)$  were inoculated directly from the swab. The plate was streaked with a sterile wire loop. Swabs were also used to inoculate tubes of SD<sup>+</sup> broth. Plates and broths were incubated at 37°C for 18 to 24 h. Colonies which developed on SD<sup>+</sup> agar were transferred to tubes of calf serum, incubated at 37°C for 1 to 2 h, and examined by phase microscopy for the presence of germ tubes. If no colonies developed on SD<sup>+</sup> agar, broth tubes were examined microscopically for the presence of yeasts; if results were positive, SD<sup>+</sup> agar was restreaked, incubated, and examined as described above.

For Vibrio isolation, swabs were used to inoculate tubes of alkaline peptone broth containing 1% Bacto-Peptone (Difco) and 1% NaCl; pH was adjusted to 8.5. After incubation at 37°C for 6 to 18 h, a loopful from turbid tubes was streaked on thiosulfate-citrate-bile-sucrose agar (Difco) which was incubated at 37°C for 18 to 24 h. Typical Vibrio colonies (yellow or blue-green) were transferred to slants of Bacto Marine Agar 2216 (Difco). Gram-negative, cytochrome oxidase-positive isolates were examined for susceptibility to 150 µg of the vibriostatic agent 0/129 (Sigma Chemical Co., St. Louis, Mo.) and reaction in glucose MOF medium (15). The API 20E system (Analytab Products, Plainview, N.Y.), with 20‰ artificial seawater as diluent, and incubation at 37°C were used to identify Vibrio species. Growth in various NaCl concentrations was used in some cases to confirm species assignment.

Table 1 shows the recovery rate of *C. albicans* and *Vibrio* spp. from birds. Compared with that in the previous study (1), the frequency of *C. albicans* recovery from gulls in Florida was similar; 41% versus 38%. Samples from Connecticut showed a 58% recovery, while a rate of 78% was noted previously. That study (1) included collections from a reservoir, docks, and an island rookery, all of which showed >80% positive samples, and this high rate of positive samples raised the overall average. No *C. albicans* were observed in pelican droppings in the present study or earlier (1), probably because pelicans are exclusively fish eaters and the incidence of pathogenic yeasts would be low in marine

<sup>&</sup>lt;sup>†</sup> Contribution no. 221 from the University of Connecticut, Marine Sciences Institute, Marine Research Laboratory, Noank, Conn.

Bird	Connecticut		Florida	
	No. positive for Vibrio spp./no. sampled (% positive)	No. positive for <i>C. albicans</i> /no. sampled (% positive)	No. positive for Vibrio spp./no. sampled (% positive)	No. positive for C. albicans/no. sampled (% positive)
Gull Pelican Cormorant Egret	23/45 (51)	26/45 (58)	29/42 (69) 20/42 (41) 1/2 (50) 1/2 (50)	17/42 (41) 0/20 (0) 1/2 (50)
Canada goose Swan	1/16 (6) 2/3 (67)	1/16 (6)		

TABLE 1. Recovery of Vibrio spp. and C. albicans from aquatic birds

fish. Isolation frequency was low from Canada geese (6%), and no yeasts were found in swans or egrets, although the number of samples from the latter two species may have been too few to be significant. *C. albicans* was found in one of two cormorant samples, but this was also statistically invalid.

The percentages of vibrio isolations from gulls in the two areas were relatively comparable (58 and 69%), and recovery from pelicans was frequent (41%). All other bird species studied also showed the presence of *Vibrio* spp., although, as discussed above, the number of samples from swans, cormorants, and egrets was low. Additional samples should be taken for a better assessment of carriage rate.

Vibrio alginolyticus was recovered from gulls, swans, and Canada geese in Connecticut and from gulls and pelicans in Florida. This is the first report of the presence of this species in aquatic birds. Vibrio parahaemolyticus was found in Connecticut gulls and from all bird species samples in Florida. Non-O1 V. cholerae was recovered from Connecticut gulls only, but the organism is apparently common in other areas (14, 16–18). Pelicans yielded isolates of both V. damsela and V. fluvialis; the latter was also found in Connecticut gulls. These two Vibrio spp. have not been noted previously in birds.

The results indicated that both *C. albicans* and several species of *Vibrio* are common in marine birds along both the northeast and southeast U.S. coastlines. The widespread occurrence of potentially pathogenic vibrios may be important to the seafood industry because of the close association of birds, particularly gulls, with fishery activities. *Vibrio* spp. occur in shellfish in different geographical areas in both natural populations (3, 13) and market samples (2, 9). Marine birds may serve as vectors or reservoirs of these pathogens (14, 23).

A portion of this work was conducted while I was on sabbatic leave at the Mote Marine Laboratory, Sarasota, Fla. I am indebted to Kumar Mahadevan, Director, and Richard H. Pierce, Associate Director, for providing facilities. James Ogg kindly shared some thoughts and data.

## LITERATURE CITED

- 1. Buck, J. D. 1983. Occurrence of *Candida albicans* in fresh gull feces in temperate and subtropical areas. Microb. Ecol. 9: 171–176.
- Chan, K., M. L. Woo, L. Y. Yam, and G. L. French. 1989. Vibrio parahaemolyticus and other halophilic vibrios associated with seafood in Hong Kong. J. Appl. Bacteriol. 66:57–64.
- DePaola, A., M. W. Presnell, M. L. Motes, Jr., R. M. McPherson, R. M. Twedt, R. E. Becker, and S. Zywno. 1983. Non-O1 Vibrio cholerae in shellfish, sediment and waters of the U.S. Gulf coast. J. Food Prot. 46:802–806.

- Farmer, J. J., III, F. W. Hickman-Brenner, and M. T. Kelly. 1985. Vibrio, p. 282-301. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
- 5. Fenlon, D. R. 1983. A comparison of salmonella serotypes found in the faeces of gulls feeding at a sewage works with serotypes present in the sewage. J. Hyg. 91:47-52.
- Fenlon, D. R. 1985. Wild birds and silage as reservoirs of Listeria in the agricultural environment. J. Appl. Bacteriol. 59:537-543.
- Fricker, C. R., and N. Metcalf. 1984. Campylobacters in wading birds (Charadrii): incidence, biotypes, and isolation techniques. Zentrabl. Bakteriol. Parsitenkd. Infektionskr. Abt. I Orig. Reihe B 179:469–475.
- Greig, S. A., J. C. Coulson, and P. Monaghan. 1986. A comparison of foraging at refuse tips by three species of gull (Laridae). J. Zool. ser. A 210:459–472.
- Hackney, C. R., B. Ray, and M. L. Speck. 1980. Incidence of Vibrio parahaemolyticus in and the microbiological quality of seafood in North Carolina. J. Food Prot. 43:769–773.
- Janda, J. M., C. Powers, R. G. Bryant, and S. L. Abbott. 1988. Current perspectives, on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. Clin. Microbiol. Rev. 1:245– 267.
- Karunasagar, I., I. Karunusagar, and C. N. Nagesha. 1987. Spontaneous mutation to 0/129 resistance in Vibrio parahaemolyticus. Curr. Sci. 56:773–775.
- Kawaoka, Y., T. M. Chambers, W. L. Sladen, and R. G. Webster. 1988. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology 163:247-250.
- Kelly, M. T., and E. M. D. Stroh. 1988. Occurrence of Vibrionaceae in natural and cultivated oyster populations in the Pacific Northwest. Diagn. Microbiol. Infect. Dis. 9:1–5.
- 14. Lee, J. V., D. J. Bashford, T. J. Donovan, A. L. Furniss, and P. A. West. 1984. The incidence and distribution of V. cholerae in England, p. 427-450. In R. R. Colwell (ed.), Vibrios in the environment. John Wiley & Sons, Inc., New York.
- Lemos, M. L., A. E. Torzano, and J. L. Barja. 1985. Modified medium for the oxidation-fermentation test in the identification of marine bacteria. Appl. Environ. Microbiol. 49:1541– 1543.
- Ogg, J. E., R. R. Ryder, and H. L. Smith, Jr. 1989. Isolation of Vibrio cholerae from aquatic birds in Colorado and Utah. Appl. Environ. Microbiol. 55:95-99.
- Roberts, N. C., H. B. Bradford, Jr., and J. R. Barbay. 1984. Ecology of Vibrio cholerae in Louisiana coastal areas, p. 389–398. In R. R. Colwell (ed.), Vibrios in the environment. John Wiley & Sons, Inc., New York.
- Schlater, L. K., B. O. Blackburn, R. Harrington, D. J. Draper, J. Van Wagner, and B. R. Davis. 1981. A non-O1 Vibrio cholerae isolated from a goose. Avian Dis. 25:199-201.
- 19. Shayegani, M., W. B. Stone, I. DeForge, T. Root, L. M. Parsons, and P. Maupin. 1986. Yersinia enterolitica and related species

isolated from wildlife in New York state. Appl. Environ. Microbiol. 52:420-424.

- Telfer, T. C., and J. K. Shisler. 1981. Record of movement of a laughing gull from Hawaii to New Jersey. J. Field Ornithol. 52:340.
- 21. Wade, J. C., and S. C. Schimpff. 1985. Epidemiology and prevention of candida infections, p. 111-133. In G. P. Bodey

and V. Fainstein (ed.), Candidiasis. Raven Press, New York. 22. Wall, T. 1983. Coots and other birds eating goose- and gull-

- Waii, T. 1965. Cools and other birds eating goose- and gundroppings. Brit. Birds 76:410–411.
  West, P. A., and J. V. Lee. 1984. Distribution and growth of
- Vibrio cholerae in a natural brackish water system, p. 451–462. In R. R. Colwell (ed.), Vibrios in the environment. John Wiley & Sons, Inc., New York.