

Occurrence and Pathogenic Role of *Morganella-Proteus-Providencia* Group Bacteria in Human Feces

HANS E. MÜLLER

Staatliches Medizinaluntersuchungsamt Braunschweig, D-3300 Braunschweig, Federal Republic of Germany

Received 5 August 1985/Accepted 28 October 1985

A total of 2,693 fecal specimens, with 1,422 from healthy persons and 1,271 from patients suffering from enteric diseases, was investigated to isolate species of the *Morganella-Proteus-Providencia* group and to evaluate the role of these bacteria in intestinal disorders. Most strains were isolated from two media, i.e., blood agar and tryptophan agar. Two of the species were more frequently found in diarrheal cases than in healthy controls. These species were *Morganella morganii* and *Proteus mirabilis*. Two new species of *Enterobacteriaceae*, i.e., *Proteus penneri* and *Providencia rustigianii*, were found in 33 and 5 people, respectively. However, these two species were not found more frequently in the diarrheal cases.

There has been some speculation in literature that organisms of the *Morganella-Proteus-Providencia* group can cause diarrhea. However, the etiological role of these bacteria has not been as firmly established as it has been for *Salmonella* and *Shigella* species. In another paper the production of brownish pigment by organisms of the *Morganella-Proteus-Providencia* group was described (H. E. Müller, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. A, in press). Aromatic amino acids are metabolized in the presence of iron ions under aerobic conditions to a water-soluble, melanin-like pigment with a variable molecular weight of less than 12,000.

The purpose of the present study was to evaluate amino acid media used to isolate the *Morganella-Proteus-Providencia* group and to determine the distribution of the species of this group in healthy people and in diarrheal cases.

Fecal specimens from healthy human beings and diarrheal cases from Braunschweig, Federal Republic of Germany, were investigated. The specimens were collected in stool preservatives and were received from healthy people employed in food processing as controls or from physicians for diagnosis.

The samples were cultivated on the following media: sheep blood agar consisting of blood agar base (CM 55; Oxoid Ltd.) and amino acid agar consisting of 5 g of proteose peptone (L 46; Oxoid), 5 g of yeast extract powder (L 21; Oxoid), 5 g of sodium chloride (no. 6400; E. Merck AG), 10 g of bacteriological agar (L 1, Oxoid), 10 g of calcium carbonate (no. 2046, Merck), 250 mg of ferric pyrophosphate (P 6526; Sigma Chemical Co.), 990 ml of distilled water, and 5 g of L-phenylalanine, L-tryptophan, or L-tyrosine as described elsewhere (Müller, in press). Incubation was at $36 \pm 1^\circ\text{C}$ for 2 days.

All colonies swarming on blood agar and all colonies showing brownish pigment on media containing an aromatic amino acid or elaborating an almondlike odor on phenylalanine agar were isolated and differentiated by standard procedures (1, 2, 8, 9).

A comparison of the strains of the *Morganella-Proteus-Providencia* group isolated from the four media is presented in Table 1. Blood agar was the best medium for detection and isolation of swarming *Proteus* sp.; 295 of 361 strains were isolated from blood agar, and 244 of 361 strains were found on tryptophan agar by means of its brownish pigment.

However, the production of brownish pigment on tyrosine agar and the pigment production together with an almondlike odor on phenylalanine agar were not very helpful in the detection or isolation of *Morganella*, *Proteus*, and *Providencia* species from heavily contaminated cultures.

Table 2 shows the spectrum of several species and biogroups in fecal specimens from 1,422 healthy people and 1,271 humans with gastrointestinal syndromes potentially of an infectious etiology.

Proteus mirabilis was found most frequently in feces of patients; it occurred more rarely in healthy persons. The difference is statistically significant ($P < 0.001$) calculated by the chi-square test with Yates' correction (15). *Morganella morganii* is also a potential pathogen. It occurred more frequently in diarrhea cases than in healthy people. The difference is also statistically significant ($P < 0.01$). The recently described *Proteus penneri* (4) was isolated a little more frequently from patients, but no statistical difference exists at the 90% confidence limit. Nevertheless, this microorganism may have disease-producing potential. Neither biogroup 2 nor biogroup 3 of *Proteus vulgaris* appeared involved with the patients studied. Furthermore, the three species of *Providencia*, i.e., *P. alcalifaciens*, *P. rettgeri*, and *P. rustigianii*, do not seem to be involved in intestinal disease production. It was surprising that no strain of *Providencia stuartii* was isolated from fecal specimens, although this species plays an important role in nosocomial and urinary tract infections. (3, 6, 7, 10, 13, 14). Therefore,

TABLE 1. Numbers of strains of the *Morganella-Proteus-Providencia* group isolated from fecal specimens and comparison of the efficiency of four media

Isolation medium	No. of strains isolated (of 361)
Sheep blood agar (total).....	295
Tryptophan agar (total).....	244
Sheep blood agar plus tryptophan agar.....	194
Sheep blood agar (only).....	101
Tryptophan agar (only).....	50
Tyrosine agar (only).....	16
Phenylalanine agar (only).....	0

TABLE 2. Occurrence of species of the *Morganella-Proteus-Providencia* group in fecal specimens of healthy people and of patients suffering from gastrointestinal syndromes

Species isolated	No. of strains isolated	Strains isolated from:		χ-Square test	
		Healthy people	Patients	Quantile	P
<i>M. morgani</i>	21	4	17	8.21	<0.01
<i>Proteus mirabilis</i>	176	38 ^a	138 ^b	58.75	<0.001
<i>Proteus penneri</i>	33	13	20	1.83	>0.1
<i>Proteus vulgaris</i>	116	61 ^c	55 ^d		
Biotype 2	78	46	32		
Biotype 3	13	7	6		
<i>Providencia alcalifaciens</i>	3	1	2		
<i>Providencia rettgeri</i>	5	3	2		
<i>Providencia rustigianii</i>	5	3	2		

^a One strain was trehalose negative, and one strain showed no motility.

^b Four strains were ornithinedecarboxylase negative, one strain was xylose negative, and one strain produced no H₂S.

^c Two strains of biotype 2 were xylose negative, and one strain of biotype 2 was sucrose negative.

^d One strain each of biotypes 2 and 3 produced no H₂S, and one strain of biotype 2 was sucrose negative.

it appears that the human intestine is an infrequent ecological niche of *P. stuartii*. Furthermore, five strains of *P. rustigianii*, a recently described species (5), were isolated. To date, only a few strains have been found in human beings; the majority of strains were isolated from penguins and were designated *Providencia friedericiani* (11). However, according to the International Code of Nomenclature of Bacteria, the name *P. rustigianii* has priority because it was published and validated before *P. friedericiani*.

It would seem that the pathogenic potentials of the several species of the *Morganella-Proteus-Providencia* group are quite different. The results of this study indicate that only *M. morgani* and *P. mirabilis* have a role in diarrheal disease. It is noteworthy that the newly described species, i.e., *Proteus penneri* (2, 4) and *Providencia rustigianii* (2, 5, 11), were isolated with some frequency. Because of the number of these strains isolated, the assumption that these hitherto unknown species, especially *Proteus penneri*, spread recently in the Federal Republic of Germany must be considered, although it is hard to accept that it would not have been detected as an atypical indole-negative *Proteus vulgaris* or an atypical ornithine decarboxylase-negative *Proteus mirabilis*. It is likely that it had been isolated just as frequently before and was overlooked or misidentified.

I thank Sabine Müller for her excellent technical assistance and David J. Williams for help in preparation of the manuscript.

LITERATURE CITED

- Cowan, S. T. 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge.
- Farmer, J. J., III, B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen-Grady, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner. 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. *J. Clin. Microbiol.* 21:46-76.
- Hawkey, P. M., J. L. Penner, M. R. Potten, M. Stephens, L. J. Barton, and D. C. E. Speller. 1982. Prospective survey of fecal, urinary tract, and environmental colonization by *Providencia stuartii* in two geriatric wards. *J. Clin. Microbiol.* 16:422-426.
- Hickman, F. W., A. G. Steigerwalt, J. J. Farmer III, and D. J. Brenner. 1982. Identification of *Proteus penneri* sp. nov., formerly known as *Proteus vulgaris* indole negative or as *Proteus vulgaris* biogroup 1. *J. Clin. Microbiol.* 15:1097-1102.
- Hickman-Brenner, F. W., J. J. Farmer III, A. G. Steigerwalt, and D. J. Brenner. 1983. *Providencia rustigianii*: a new species in the family *Enterobacteriaceae* formerly known as *Providencia alcalifaciens* biogroup 3. *J. Clin. Microbiol.* 17:1057-1060.
- Kocka, F. E., S. Srinivasan, M. Mowjood, and H. S. Kantor. 1980. Nosocomial multiply resistant *Providencia stuartii*: a long-term outbreak with multiple biotypes and serotypes at one hospital. *J. Clin. Microbiol.* 11:167-169. (Erratum 12:295.)
- Kopf, P.-O., and V. Freitag. 1979. Krankenhausinfektionen mit *Providencia stuartii*. *Dtsch. Med. Wochenschr.* 104:1129-1132.
- Lenette, E. H., A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.). 1985. Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
- MacFaddin, J. F. 1981. Biochemical tests for identification of medical bacteria, 2nd ed. The Williams & Wilkins Co., Baltimore.
- Müller, H. E. 1981. *Providencia*, p. 94-99. In H.-G. Sonntag und H. E. Müller (ed.), *Infektionserreger in Praxis und Krankenhaus*. I. mhp-Verlag GmbH, Mainz, Federal Republic of Germany.
- Müller, H. E. 1983. *Providencia friedericiani*, a new species isolated from penguins. *Int. J. Syst. Bacteriol.* 33:709-715.
- Penner, J. L. 1981. The tribe *Proteae*, p. 1204-1224. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), *The prokaryotes*, vol. 2. Springer-Verlag, Berlin.
- Penner, J. L., N. A. Hinton, I. B. R. Duncan, J. N. Hennessy, and G. R. Whiteley. 1979. O serotyping of *Providencia stuartii* isolates collected from twelve hospitals. *J. Clin. Microbiol.* 9:11-14.
- Prentice, B., and B. L. Robinson. 1979. A review of *Providencia* bacteremia in a general hospital, with a comment on patterns of antimicrobial sensitivity and use. *Can. Med. Assoc. J.* 121:745-749.
- Walter, E. 1975. *Biomathematik für Mediziner*. B. G. Teubner, Stuttgart, Federal Republic of Germany.