Rare occurrence of *Proteus vulgaris* in faeces: a reason for its rare association with urinary tract infections

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Summary. The faecal carriage rates of different species of *Proteeae* were assessed in studies with 220 faecal isolates from 219 individuals of whom approximately one-third were well and the remainder had gastro-enteritis. As a result of the development of new media that allowed replacement of the phenylalanine deaminase test with the tryptophan deaminase test and made it possible to combine tests for indole and urease production and for hydrogen sulphide and ornithine decarboxylase formation in two single-tube tests, all strains were speciated with speed, economy and accuracy. Most (96%) isolates were either *Proteus mirabilis* (62%) or *Morganella morganii* (34%). The significance of these findings in relation to urinary tract infection is discussed. *P. vulgaris* was found in only one (0.45%) faecal specimen and this rarity of carriage in faeces is believed to be the main reason for its rare association with urinary tract infections. The frequent association of *M. morganii*, in the absence of other enteropathogenic bacteria, with severe gastroenteritis was noted with interest.

Introduction

Although infections of the urinary tract with strains of *Proteus* occur less frequently than those with *Escherichia coli*, they are particularly important in young boys (Bergström, 1972; Hallett *et al.*, 1976) and the elderly (Walkey *et al.*, 1967; Senior, 1979). Furthermore, the consequences of infection with *Proteus* are often more serious than those with *E. coli*, which is usually confined to the bladder, for *Proteus* has a special predilection for the upper urinary tract (Fairley *et al.*, 1971; Svanborg Edén *et al.*, 1980) where it may cause damage and death to the renal tubular epithelium (Braude *et al.*, 1960) and the formation of renal stones (Griffith *et al.*, 1973). These events arise through the formation by *Proteus* species of potent urease isoenzymes (Senior *et al.*, 1980) which cause the degradation of urea and rapidly make the urine alkaline (Senior, 1983).

The common source of urinary tract pathogens is the bowel. Most infections of the urinary tract with *Proteus* are due to *P. mirabilis* (96.5%) and very few to *P. vulgaris* (1.5%) or *Morganella morganii* (*P. morganii*) (1.5%) (Senior, 1979). DNA hybridisation studies (Brenner *et al.*, 1978), urease isoenzyme profiles (Senior *et al.*, 1980) and the sharing of phage (Coetzee, 1963) and bacteriocin receptors

(Senior and Larsson, 1983) all indicate the close relationship between *P. mirabilis* and *P. vulgaris*. It is remarkable, therefore, that there should be such a wide difference in the frequency with which these organisms are associated with urinary tract infections.

One explanation for the rare association of *P. vulgaris* with infections of the urinary tract might be the fact that analogous bacteriocin production/sensitivity (P/S) types to those associated with *P. mirabilis* strains having special affinity for the urinary tract (Senior, 1979) have not yet been found in *P. vulgaris* (unpublished results). Alternatively, there may be a much simpler explanation based on the frequency of occurrence of *P. vulgaris* in the faeces. The aim of this study was to investigate the latter theory through the development of new multi-combination biochemical tests which would permit the identification of all species of members of the tribe *Proteeae* to be made with speed, ease, economy and accuracy.

Materials and methods

Source and isolation of bacterial strains

Strains belonging to the tribe *Proteeae* were isolated from specimens of human faeces submitted to the laboratory for routine investigations. A loopful of each specimen was plated out for single colonies on Desoxycholate

Citrate Agar (DCA; Oxoid CM35) and also inoculated into Tetrathionate Broth (Oxoid CM29). Both media were incubated overnight at 37°C. The tetrathionate-broth culture was plated out for single colonies on a DCA plate which was incubated overnight at 37°C. One non-lactose-fermenting (NLF) colony of every morphological type present on the DCA plates was picked and inoculated into Nutrient Broth (Oxoid CM67). After overnight incubation at 37°C a loopful of each broth culture was placed on the surface of a dried tryptophan deaminase agar plate and a loopful seeded into urea/indole medium. After overnight incubation at 37°C, those cultures that produced tryptophan deaminase were saved and subsequently speciated as detailed below.

Media

All commercially prepared media were dispensed and sterilised according to the manufacturer's instructions. Each batch of every medium was tested with appropriate positive and negative control test organisms before being used in the study. *Tryptophan deaminase agar medium* (TDA) contained bacteriological peptone (Oxoid L37) 30 g, Lab-Lemco powder (Oxoid L29) 50 g, L-tryptophan (Sigma) 10 g, Agar No. 3 (Oxoid L13) 12 g, bile salts (Oxoid L55) 3 g and yeast extract (Oxoid L21) 3 g dissolved in distilled water to a volume of 1 L. The medium was sterilised by autoclaving at 121°C for 15 min and, when cool, poured into petri dishes. *Urea/indole medium* was prepared by supplementing 95 ml of sterile Tryptone Water (Oxoid CM87) with 5 ml of a Seitz-filtered refrigerated stock solution of urea 40 g, phenolphthalein (1% w/v in propan-2-ol) 10 ml made up to 100 ml with distilled water. The medium was dispensed into sterile, cotton wool-plugged tubes. *Ornithine decarboxylase/sulphide medium* contained Tryptone Water (Oxoid CM87) 0.5 g, L-ornithine hydrochloride (Sigma) 1 g, ferrous ammonium sulphate 20 mg, sodium thiosulphate 20 mg and a little bromocresol purple dye, dissolved in distilled water to 100 ml. After autoclaving at 121°C for 15 min, the medium was allowed to cool and then supplemented with glucose to a final concentration of 0.1% w/v from a Seitz-filtered glucose 10% w/v in water stock solution. The medium was dispensed aseptically into bijoux bottles. *Triple sugar agar medium* was prepared by supplementing sterile molten, cool Lab-Lemco Agar (Oxoid CM17) containing neutral red with lactose, maltose and inositol, each to a concentration of 1% w/v from Seitz-filtered 10% w/v stock solutions of each sugar in water. After gentle mixing, the medium was poured into petri dishes. *Peptone water sugars* were made by supplementing Peptone Water (Oxoid CM9) with a little bromocresol purple dye and the sugar to a final concentration of 1% w/v. The medium was dispensed into tubes or bijoux bottles and sterilised by steaming for 30 min on each of three successive days.

Speciation of strains

The formation of tryptophan deaminase was indicated

by the formation of a stable brown pigment around strains growing on TDA plates. Ornithine decarboxylase/sulphide medium and triple sugar medium were seeded from broth cultures of all strains that formed tryptophan deaminase. Those strains which failed to produce urease as indicated by an inability to turn the pH indicator in the urea/indole medium to pink were, in addition, inoculated from their broth cultures into glucose, galactose, and trehalose peptone-water sugars. All inoculated media were incubated overnight at 37°C. Ehrlich's reagent was gently added to the cultures in the urea/indole medium. Indole formation was indicated by the reagent turning red. Ornithine decarboxylase formation was indicated by the development of alkalinity. Hydrogen sulphide production was revealed by the formation of a black deposit. If any isolate formed acid on the triple sugar agar medium, four tubes of single sugar peptone waters (lactose, maltose, inositol and adonitol) were inoculated from its broth culture, incubated overnight at 37°C and the reactions to the individual sugars recorded. The species of each isolate was determined by reference to table I.

Results

During a 6-month period, 220 strains belonging to species of the tribe *Proteeae* were isolated from the faeces of 219 people of whom 120 (55%) were female, 94 (43%) male, and five (2%) of unstated sex; 70% of the females and 60% of the males were in hospital. Clinical information was given about 202 of the individuals and of these, 148 (73%) had symptoms of gastro-enteritis in the form of diarrhoea only (72% of cases) or diarrhoea and vomiting (23% of cases) or vomiting only (5% of cases). None was receiving antibiotics when faecal samples were collected. The remaining 71 people (32% of the total) were either healthy, or unwell with undisclosed illness. Known intestinal pathogenic bacteria were found in only 20 (9%) of the specimens of faeces and these and their association with species of *Proteeae* are presented in table II.

Speciation of the 220 strains of *Proteeae* isolated showed that 136 (61.8%) were *P. mirabilis*, 74 (33.6%) were *M. morgani*, four (1.8%) were *Providencia alcalifaciens*, three (1.36%) were *Prov. rettgeri*, two (0.9%) were *Prov. stuarti* and one (0.45%) was *P. vulgaris*. There were no isolations of *Tatumella ptyseos* (Hollis *et al.*, 1981) or *Prov. rustigiani* (Hickman-Brenner *et al.*, 1983) or *P. penneri* (Hickman *et al.*, 1982).

An analysis of the frequency of isolation of the different species of *Proteeae* from the faeces of patients of different age and sex is presented in table III. *P. mirabilis* and *M. morgani* were isolated from patients in every 10-year age group from infancy to old age. Generally *P. mirabilis* was isolated a little

Table I. The biochemical reactions of members of the tribe *Proteeae* and *Tatumella ptyseos*

Organism	Biochemical test and result*											
	Tryptophan phenylalanine deaminase	Urease	Indole	Ornithine decarboxylase	H ₂ S	Lactose	Maltose	Acid from				
								Inositol	Adonitol	Trehalose	Galactose	Gas from glucose
<i>Proteus mirabilis</i>	+	(+)	-	+	(+)	-	-	-	-	+	NT	(+)
† <i>Proteus penneri</i>	+	+	-	-	V	-	+	-	-	+	+	V
<i>Proteus vulgaris</i>	+	+	+	-	(+)	-	-	-	-	V	NT	(+)
<i>Morganella morganii</i>	+	+	+	(+)	-	-	-	-	-	V	NT	(+)
<i>Providencia rettgeri</i>	+	+	+	-	-	-	-	+	+	-	+	(-)
<i>Providencia stuartii</i>	+	V	+	-	-	-	-	[+]	[-]	+	+	-
<i>Providencia alcalifaciens</i>	+	-	+	-	-	-	-	-	+	-	-	V
‡ <i>Providencia rustigiani</i>	+	-	(+)	-	-	-	-	-	-	-	+	V
§ <i>Tatumella ptyseos</i>	+	-	-	-	-	-	-	-	-	+	+	-

* + = Production; - = no production; () = most strains; V = different strains give different results; NT = not tested.

† Data from Hickman *et al.*, 1982.

‡ Data from Hickmann-Brenner *et al.*, 1983.

§ Data from Hollis *et al.*, 1981.

Table II. The 20 enteropathogenic isolates* and their association with species of the tribe *Proteeae*

Enteropathogenic organism	Number of isolations of pathogen				
	in association with				
	Total	<i>P. mirabilis</i>	<i>M. morganii</i>	<i>Prov. rettgeri</i>	<i>P. vulgaris</i>
<i>Salmonella</i> sp.	10	5	4	1	0
<i>Shigella</i> sp.	3	0	2	0	1
<i>Campylobacter</i> sp.	3	1	2	0	0
<i>Clostridium difficile</i>	3	2	0	1	0
Non-cholera vibrio	1	0	1	0	0

* The 20 enteropathogenic isolates were from 219 faecal specimens examined (see text).

more frequently than *M. morganii* regardless of the age or sex of the patient. From elderly women, *P. mirabilis* isolates greatly outnumbered *M. morganii* isolates, but the reverse situation was found in males of 11-30 and 41-50 years.

An analysis of the data with regard to clinical symptoms showed that of the 71 patients with no

symptoms of gastro-enteritis, 44 (62%) were infected with *P. mirabilis* and 27 (38%) with *M. morganii*. A similar ratio between *P. mirabilis* and *M. morganii* was found among the 107 patients with diarrhoea alone, of whom 69% were infected with *P. mirabilis* and 30% with *M. morganii*, but a different ratio was found for the 34 patients with both

Table III. Frequency of isolation of species of *Proteeae* from faeces of patients according to age and sex

Age group (years)	Number of strains of the stated species													
	from male patients							from female patients						
	Number of patients	<i>P. mirabilis</i>	<i>M. morgani</i>	<i>P. vulgaris</i>	<i>Prov. rettgeri</i>	<i>Prov. alcalifaciens</i>	<i>Prov. stuarti</i>	Number of patients	<i>P. mirabilis</i>	<i>M. morgani</i>	<i>P. vulgaris</i>	<i>Prov. rettgeri</i>	<i>Prov. alcalifaciens</i>	<i>Prov. stuarti</i>
0-10	44	27	17	0	0	0	0	36	23	11	0	1	1	0
11-20	2	0	2	0	0	0	0	9	5	3	1	0	0	0
21-30	9	1	8	0	0	0	0	11	5	6	0	0	0	0
31-40	6	4	2	0	0	0	0	3	3	0	0	0	0	0
41-50	5	1	4	0	0	0	0	9	5	2	0	0	2	0
51-60	4	2	1	0	0	1	0	3	2	1	0	0	0	0
61-70	2	2	0	0	0	0	0	9*	7	2	0	0	0	1
71-80	7	4	2	0	0	0	1	15	13	1	0	1	0	0
81+	6	5	1	0	0	0	0	19	15	4	0	0	0	0
Unknown	9	4	4	0	1	0	0	6	5	1	0	0	0	0
Total	94	50	41	0	1	1	1	120	83	31	1	2	3	1
Percentage of total	100	53	44	0	1	1	1	100	70	26	0.8	1.6	2.4	0.8

There were 5 patients of unstated age and sex of whom 3 yielded *P. mirabilis* and 2 yielded *M. morgani*.

* One patient carried 2 different species.

diarrhoea and vomiting among whom 50% were infected with *P. mirabilis* and 47% with *M. morgani*.

Discussion

This study sought the development of new media to permit the biochemical speciation of all representatives of the tribe *Proteeae* with speed, economy of media and the minimum of manipulations in order that the degree of correlation between frequency of faecal carriage of the different species and their frequency in urinary tract infection might be assessed.

The new media that we developed proved reliable and are believed to be accurate; with very few exceptions, each strain gave results that were typical and fully in agreement with only one of the different species of the tribe *Proteeae*. The exceptions included a small number of H₂S-negative variants of *P. mirabilis* and *P. vulgaris*, well within the frequency range observed by others (Brenner *et al.*, 1978), and one strain of the ornithine decarboxylase-negative biotype of *M. morgani* which others have described (Hickman *et al.*, 1980).

Strains belonging to the tribe *Proteeae* are easily recognised for, with the exception of strains of *Tatumella pytyseos* (Hollis *et al.*, 1981), they are unique among the Enterobacteriaceae in possessing the ability to deaminate amino acids oxidatively. The test for this ability has usually been performed with phenylalanine, the deaminated product phenyl pyruvic acid forming a transient green colour upon addition of ferric chloride (Henriksen and Closs, 1938; Henriksen, 1950). Polster and Svobodova (1964) advocated the use of tryptophan instead of phenylalanine and reported that only strains of the tribe *Proteeae* formed a red-brown diffusible pigment (presumably indole-3-pyruvic acid) when growing on media containing tryptophan. Using their medium, we found, as they did, that a small number of *M. morgani* strains failed to produce pigment. The modified medium we devised supported a better growth of *M. morgani* and all strains of *Proteeae* produced pigment. All non-pigment-forming strains were also phenylalanine deaminase negative (data not shown). The inclusion of bile salts appeared to have no effect on pigment production but, by preventing swarming, permitted about

12 strains to be tested together on one plate. The brown pigment, in contrast to the green pigment from phenylalanine, did not fade nor did it require the addition of ferric chloride to show its presence. Because of these factors and the ease, reliability and reproducibility of the test, we recommend the use of our modified tryptophan deaminase medium in preference to the phenylalanine test.

The urea/indole medium proved helpful in that it permitted rapid recognition, with high probability, of the commonest species of *Proteaeae*, *P. mirabilis* (urease positive, indole negative), in a single tube test. Phenolphthalein was selected as pH indicator instead of the more commonly used indicator phenol red because it changed from colourless to pink at a much higher pH than the latter, thereby avoiding possible confusion between weak urease producers, e.g. strains of *Enterobacter*, *Serratia* and *Pseudomonas* species, and the strong urease producers of the *Proteaeae*. The tryptone water was sufficiently buffered and rich to permit good growth and tryptophan breakdown before high alkalinity prevented this. There was never any difficulty in reading both urease positive and indole positive reactions because one was pink and the other red and the acid of the Ehrlich's reagent caused a yellow band to appear between each colour.

Detection of hydrogen sulphide formation is often not very reliable but the ornithine decarboxylase/sulphide medium we devised was always satisfactory and most useful in that the reactions in this one medium when considered with the indole/urea result could distinguish four different species—*P. mirabilis*, *P. vulgaris*, *M. morgani* and *Prov. rettgeri*.

In our experience, strains of *P. mirabilis* and *M.*

morgani are more frequently encountered than *P. vulgaris* and *Providencia* species and with this in mind our triple sugar medium fulfilled two useful functions. If strains did not show acid formation on it they were pure isolates of either *P. mirabilis* or *M. morgani* and free from lactose-fermenting contaminants. Whenever a strain formed acid on the medium this indicated that the isolate was either a lactose-fermenting contaminant or a strain of the more rarely encountered *P. vulgaris*, *P. penneri* or a species of *Providencia*, and this was readily resolved to species level by subculture in the four single-sugar tubes.

The most commonly isolated species of *Proteaeae* from faeces was *P. mirabilis* and this would explain its frequent association with urinary tract infections (Senior, 1979). *M. morgani* was also common and found in about one third of faecal specimens irrespective of the age and sex of the patient. Its rare association with urinary tract infections (Senior, 1979) has been attributed to its relatively slow growth rate in urine and its inability to make urine rapidly alkaline despite its potent urease enzyme (Senior, 1983). The results showed unequivocally that *P. vulgaris*, in spite of its name (*vulgaris* = common), was rarely found in human faeces and this is therefore the most probable reason for its rare association with urinary tract infections (Senior, 1979).

It was noted with interest that, in the absence of other known enteropathogenic bacteria or antibiotic therapy, *M. morgani* was associated with diarrhoea and vomiting at a higher frequency than expected. We hope to investigate whether such strains can produce enterotoxins.

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