Speciation, serotyping, antimicrobial sensitivity and plasmid content of Proteeae from the environment of calf-rearing units in South West England

By P. M. HAWKEY*, J. L. PENNER†, A. H. LINTON*, C. A. HAWKEY*,
L. J. CRISP† AND M. HINTON‡

** Department of Microbiology, University of Bristol, University Walk,
Bristol BS8 TD1, England † Department of Microbiology, University of
Toronto, Banting Institute, 100 College Street, Toronto, Ontario MG5 1L5,
Canada, and ‡ Department of Veterinary Medicine, University of Bristol,
Langford House, Langford, Avon BS18 7DU, England*

SUMMARY

A survey was undertaken of the occurrence, serotype, antimicrobial sensitivity and plasmid content of members of the tribe Proteeae in the environment of two calf-rearing units in the county of Avon in South West England. Examples of the following species were found: *Proteus mirabilis*, *Prot. vulgaris*, *Prot. vulgaris* Biogroup 2, *Morganella morganii*, *Providencia stuartii*, *Prov. alcalifaciens* and *Prov. rettgeri*. A wide range of serotypes was found, many having been previously reported from nosocomial isolates. A total of 15% of isolates carried plasmids; six pairs of isolates were identified which had identical serotypes but different patterns of plasmid carriage. The antimicrobial sensitivity of the isolates was generally similar to isolates of Proteeae from humans. Although no truly aminoglycoside-resistant isolates were found, some isolates of *Prov. stuartii* and *Prov. rettgeri* had MIC's higher than the other isolates to gentamicin and netilmicin, suggesting the presence of low levels of the enzyme AAC 2′. The study demonstrates that there is a considerable diversity of species and types of Proteeae associated with calves and their environment. It seems likely that a potential cause of colonization of the human gut by Proteeae is the consumption of meat.

INTRODUCTION

The widespread distribution of R factors in salmonellae and *Escherichia coli* isolated from food animals is well documented (Anderson, Humphreys & Threlfall, 1975; Threlfall & Rowe, 1984; Linton & Hinton, 1984; Hirsch *et al.* 1983). Many of these R factors mediate resistance to antibiotics commonly used to treat human infections and in turn humans may acquire bacteria possessing these factors by eating contaminated foods (Linton, 1977; Linton *et al.* 1977). Although this has been demonstrated to occur with *E. coli* and salmonellae, it may be presumed to occur with other members of the Enterobacteriaceae that colonize the human gut and are also found in food animals.

Bacteria of the tribe Proteeae are a common cause of hospital-acquired infections, particularly urinary tract infections (Meers *et al.* 1981). Faecal colonization of patients by Proteeae has been described in most of the species of Proteeae (Chow *et al.* 1979; Toni, Casewell & Schito, 1980; Hawkey *et al.* 1982). Patients with faeces that are colonized by Proteeae are also known to be sources of nosocomial infection (Chow *et al.* 1979; Hawkey *et al.* 1982). It therefore seems probable that the human gut may be colonized by Proteeae that have been ingested at some time. This event may occur during an admission to hospital as a consequence of nosocomial cross-infection or before admission, resulting in the organism being introduced to the hospital environment. Proteeae acquired via food may, as with *E. coli* and salmonellae, carry R factors, thus introducing new resistance genes into the human gut flora from the animal ecosystem. Although much is known about the occurrence and characteristics of salmonellae and *E. coli* in food animals and their environment, very little is known about Proteeae in this setting.

METHODS

Sampling protocol

Two farms in the County of Avon area in South West England were studied. On one farm (Farm L) samples were taken over a total period of 13 weeks; between 1 and 3 samples of bedding contaminated with faeces and urine being taken from yearling calf pens for the first 10 weeks, 2 calf pens for the last 2 weeks (variation in the number of samples taken is related to variations in the occupancy of pens) and 3 milk filters for 1 week. The second site (Farm W) was studied extensively over 2 weeks, 6 samples of bedding contaminated with excreta being taken each week.

Bacterial isolation and identification procedures

Bedding samples consisting of approximately 1 g of material were incubated at 37 °C overnight in 10 ml of Peptone water (Oxoid Ltd, Basingstoke, England) and violently agitated on a 'Rotamixer', (Hook and Tucker Instrument Ltd., Streatham, London, England) for 2 min.

Fifty microlitres of each sample were plated on to bile lactose agar (Oxoid CM7b) plates and bile lactose agar plates containing 50 mg/l ampicillin, 50 mg/l chloramphenicol and 50 mg/l tetracycline respectively and incubated for 20 h at 37 °C in air. A total of 10 lactose fermenting and 10 non-lactose fermenting colonies were selected from the non-selective plates and 5 non-lactose fermenting and 5 lactose fermenting colonies from each antibiotic containing medium. The milk filters were similarly processed.

All isolates, which were aerobic Gram-negative bacilli, positive in a liquid test medium for phenylalanine deaminase and oxidase-negative were subjected to the following biochemical tests: the ability to produce acid from adonitol, arabinose, D-arabitol, meso-erythritol, glucose, meso-inositol, D-mannitol, D-mannose, maltose, rhamnose, salicin, sucrose, trehalose and D-xylose. The ability to produce indole, utilize citrate as a sole carbon source, hydrolyse urea and aesculin, decarboxylate ornithine and deaminate phenylalanine was also tested. Species were identified on the basis of reactions described by Penner (1981), *Providencia*

rettgeri biogroups according to Penner, Hinton & Hennessy (1975) and biogroups of the *Proteus vulgaris* complex according to Hickman *et al.* (1982).

Serotyping

The O-serotyping of isolates was by the slide agglutination and passive haemagglutination system as has been previously described for *Prot. vulgaris* and *Prot. mirabilis* (Penner & Hennessy, 1980), *Morganella morganii* (Penner & Hennessy, 1979*b*), *Prov. rettgeri* (Penner, Hinton & Hennessy, 1974), *Prov. stuartii* (Penner *et al.* 1976) and *Prov. alcalifaciens* (Penner *et al.* 1979*a*).

Antibiotic sensitivity testing

Minimum inhibitory concentrations (MICs) of the antimicrobials were determined by an agar plate incorporation method described previously (Hawkey *et al.* 1982).

Isolation of plasmid DNA

A rapid, micro-method was used to isolate plasmid DNA which has been described previously (Hawkey, Bennett & Hawkey, 1984). Plasmid molecular weights were determined by electrophoresis of purified DNA in an agarose gel with plasmids of known size (pACYCl84, 3·9 kb; R388, 34·7 kb; pUB307, 54·0 kb; pUB918, 76·5 kb; Rl 101·7 kb). Strains positive for the presence of plasmids were repeated on at least three separate occasions to confirm their presence and improve the accuracy of sizing. Each batch of determinations included a strain of *Prov. stuartii* containing a well-characterized plasmid to act as an internal control (Hawkey, Bennett & Hawkey, 1985).

RESULTS

Distribution of Proteeae isolates in calf-rearing units

The occurrence of the various species of Proteeae found in this study are shown in Table 1. There are differences in the number and range of Proteeae isolated between the two farms L and W, but it must be remembered the sampling on farm W was only undertaken on 2 consecutive weeks. This may acount for the failure to isolate any *Prot. mirabilis*. However, it is interesting that, with the exception of *Prot. mirabilis*, the two most commonly isolated species (*Prot. vulgaris* BG3 and *Prov. rettgeri*) were the same from both farms, suggesting that these species are the predominant Proteeae in the faecal flora of calves. Isolates of *Prov. rettgeri* were all biotyped and the results are shown in Table 2. In contrast to biogroup distributions seen by Penner *et al.* (1975), who reported only three strains (0·4%) among their 729 isolates belonging to biogroup 4, the *Prov. rettgeri* from the farms showed a surprisingly high proportion (37%) belonging to this biogroup. The distribution of *Prot. vulgaris* biogroups was also a little unexpected; no examples of biogroup 1 (designated *Prot. penneri*) were found. The material from farm L yielded moderate numbers of both *Morganella morganii* and *Prov. stuartii*, both species have been reported as causes of nosocomial infection in man and accounts of environmental isolation of these species are few (Williams *et al.* 1983; Hawkey, 1984). The single isolate of *Prov. alcalifaciens* is unusual as this species has been

Table 1. *Distribution according to species of* 127 *Proteeae isolated from two calf-rearing units*

Species*	Total no. of isolates	No. of isolates from calf-rearing units	
		Farm L	Farm W
Prot. vulgaris			
biogroup 3	34	16	18
biogroup 2	5	2 (1)†	2
Prot. mirabilis	28	27 (1)†	0
Prov. rettgeri	35	30	5
Prov. stuartii	14	14	0
Prov. alcalifaciens	1	1	0
M. morganii	10	9	1

* Isolates of the recently defined species, *Proteus penneri* and *Providencia rustigianii* were not found.
† Number of isolates from milk filters indicated in parentheses.

Table 2. *Biogroups of* Providencia rettgeri *isolated from the two calf units*

Biogroup*	No. of isolates
1a (sal+rha−ery+)	3
1b (sal+rha−ery−)	3
2a (sal+rha+ery+)	0
2b (sal+rha+ery−)	0
3a (sal−rha+ery+)	7
3b (sal−rha+ery−)	10
4a (sal−rha−ery+)	3
4b (sal−rha−ery−)	9

* Biogroups according to Namioka & Sakazaki (1958) as modified by Penner *et al.* (1975). Abbreviations: sal, salicin; rha, rhamnose; ery, erythritol

implicated as a cause of neonatal diarrhoea in calves and more isolations might have been expected. (Waldhalm, Meinershagen & Frank, 1969).

Plasmid isolation and O-serotyping of Proteeae isolated from calf rearing farms

As can be seen from Tables 3 and 4, 14% of Proteeae from farm L and 19% of Proteeae from farm W carried one or more plasmids. Whilst it is recognized that rapid plasmid isolation methods may miss some plasmids and multiple isolates may bias figures, it is quite a high rate of carriage of plasmids.

It is interesting to note that the six isolates of Proteeae of the same serotype isolated at the same time contained different patterns of plasmids. Although one of this group of isolates, *Prot. vulgaris* non-typable from farm W week 1, may not be identical because it failed to serotype, the other five had identical serotypes, including low-titre reactions determined by passive haemagglutination. Only 11 of the 127 isolates proved to be untypable within the schemes for serotyping Proteeae used. This demonstrates the applicability of these schemes, which were largely developed using human isolates to animal studies. A rather unusual finding

Table 3. *O-serotypes and plasmids of 101 Proteeae isolated during weeks 7–13 from soiled bedding and milk filters at calf-rearing site L*

Species	O-serotypes[a] and plasmids[b] of Proteeae isolated during weeks:						
	7	8	9	10	11	12	13
Prot. vulgaris	—[c]	23 (2)[d] 23 [10,15]^b 25 NT	4; 12/42[e]; 46 (7);	12/42^e1	1[e]; 44 [7,6]; HSC 863[f]	—	—
Prot. mirabilis	17/35 (3) NT	—	103 [8,10] 16/18 (5); 16/18 [10]; 20; 20 [38]; 40; 205/7 (2)	—	16/18 (5); 40/14; 205/7/11	205/7/11	30 (4); 205/7/11
Prot. rettgeri	—	—	46; 58/76 [76,65] (2); 86; 91; 92 [45]; NT	24/41/81[i]	5; 8 (2); 10/34 [36]; 41,46; 15/57 [28]; 52 [50]; 58/76 [35]; 58/76 (4); 72; 72/75; 86 (2); 92; NT 4a[f] (2); NT 3b[j]	—	74
Prov. stuartii	25	—	52	—	1/21^g,h(4); 11^g [28]; 25 (4); 31^g; 52; 57	—	—
Prov. alcalifaciens	—	—	46	—	—	—	—
M. morganii	—	—	40	—	5 (4); 5 [36]; 5/26 (2)	—	14

[a] Italic indicates O antisera which agglutinated antigenic preparation of isolate. Isolates not typable indicated NT.
[b] Plasmids indicated in kilobases in square brackets and bold type.
[c] No isolates. (No isolates were obtained during first 6 weeks of study.)
[d] Number of isolates if more than one of the same serotype or same serotype and plasmids indicated in parentheses.
[e] Isolates of *Prot. vulgaris* biogroup 2. All other *Prot. vulgaris* isolates are biogroup 3.
[f] HSC863 is a new serotype for which a numerical designation has not been assigned.
[g] *Prov. stuartii* isolates that agglutinated in O-antisera prepared against *Prov. alcalifaciens* serostrains.
[h] Isolates of *Prov. stuartii* serotype O: 1/21 were urease-positive.
[i] Isolates obtained from milk filters.
[j] Biotype of non-typable isolates to show isolation of more than one strain.

Table 4. *Serotypes of 26 Proteeae isolated from soiled bedding samples at site W over a 2-week study period*

	1	2
Prot. vulgaris	19 (2)[a]; 32(2); 37/46 NT; NT **[8]**[b]	12/42[e] (2); 19 (2); 21; 25 (4); 37/46; NT(3)
Prov. rettgeri	—	46; 73 **[74]** (4)
M. morganii	—	5 **[9]**

[a] Underscoring indicates O antisera which agglutinated antigenic preparation of isolate. Isolates not typable indicated NT.

[b] Plasmids indicated in kilobases in square brackets and bold type.

[e] Isolates of *Prot. vulgaris* biogroup 2. All other *Prot. vulgaris* of biogroup 3.

in the *Prov. stuartii* serotyping was that two sets of strains typed only in antisera raised against *Prov. alcalifaciens*. The two milk-filter isolates most probably reflect faecal contamination of milk and indeed the *Prot. vulgaris* 0:12/42 is of the same serotype as a strains obtained from bedding. The urease-positive *Prov. stuartii* all belonged to one serotype and lacked plasmids. This finding agrees with another study. (Hollick *et al.* 1984).

Antimicrobial susceptibility

The results of the agar plate incorporation MIC determinations are shown in Table 5. All isolates of *Prot. vulgaris* and *M. morganii* were highly resistant to ampicillin, *Providencia* spp., with the exception of *Prov. alcalifaciens*, were moderately resistant, and all *Prot. mirabilis* were sensitive. No resistance to ciprofloxacin or ceftazidime was detected, whereas resistance to tetracycline, sulphonamides and cephazolin was common amongst many of the species.

DISCUSSION

Information about the species and serotype of Proteeae from non-clinical sources is almost entirely derived from early surveys or when strains were collected to establish serotyping schemes (Levine, 1942; Phillips, 1955; Ewing, Tanner & Dennard, 1954). The realization that some members of the tribe Proteeae (particularly *Prov. stuartii* and *Prov. rettgeri*) may be highly resistant to antimicrobials and cause episodes of cross-infection in hospitals has prompted interest in the epidemiology and natural distribution of these organisms (Hawkey, 1984; Stickler, Fawcett & Chawler, 1985). In the study reported by Stickler and his colleagues, hospital patients and healthy adults were examined for urinary and faecal carriage of *Prov. stuartii* (sewage and polluted river water being examined as well) (Stickler, Fawcett & Chawler, 1985). No *Prov. stuartii* were found in the sewage, water or healthy volunteers. However, the number of samples examined was low and, in view of early data on *Providencia* spp. faecal carriage (rates as low as 2 % have been reported in healthy children), the failure to find *Prov. stuartii* was not unexpected. (Singer & Bar-Chay, 1954). The data presented in this paper represents the first detailed, prospective study of Proteeae in the farm environment in recent years, when antibiotic usage has been considerable. If food animals are to be regarded as a possible natural habitat from which the human gut may be

colonized by Proteeae (albeit at a low frequency) then species and serotypes which occur commonly in humans might be expected to occur in animal faeces. With the exception of the recently recognized species *Prot. penneri*, we have found examples of all of the Proteeae currently known to cause infection in man in the farm environment. This striking diversity of species and serotypes of Proteeae has, as far as we know, not been previously reported (see Tables 3 and 4).

Numerically the largest number of isolates belonged to the *Prot. vulgaris* complex of species (biogroup 2 and 3 only). *Prot. vulgaris* has recently been split into three distinct species: *Prot. vulgaris*, *Prot. vulgaris* Biogroup 2 (to be named) and *Prot. penneri* (Hickman *et al.* 1982).

Only five isolates of *Prot. vulgaris* biogroup 2 were found, which were all serotype O: 1 or O: 12/42. It is of interest that isolates of O: 12/42 were found on successive weeks at site L and site W. In a recent study of serotypes of the *Prot. vulgaris* complex in Bristol hospitals two patients were found with urinary-tract infection caused by isolates of the same serotype (manuscript in preparation). When considering *Prot. vulgaris* (biogroup 3) the number of non-typable isolates is low (6 of 34 isolates), and as the typing scheme used (Penner & Hennessy, 1980) was based on strains from humans there seems to be a good correlation between the human and calf strains. In a study of hospital isolates of *Prot. vulgaris* in Canada, 3 of the 5 most common serotypes are found amongst our calf isolates and, disregarding non-typables, all but three serotypes from calves were also reported from hospitals in the Canadian study (Penner & Hennessy, 1980),

The next most commonly isolated member of the Proteeae was *Prov. rettgeri*, and as with *Prot. vulgaris* a great diversity of biogroups (6 of the 8 known) and serotypes (16) was recorded. Twelve of the 16 serotypes found in this study have been reported from hospitals in Canada and only 4 calf serotypes were previously reported to be associated with an environmental source (frogs) (Penner & Hennessy, 1979a). Penner & Hennessy suggested that, because of the fairly high correlation of serotypes from the aquatic environment and hospital isolates, the environment could act as a reservoir. Our study shows a higher degree of correlation of serotypes, and as the calves are ultimately destined for human consumption, faecal contamination of the meat could well explain the colonization of the human gut with *Prov. rettgeri*. The isolates belonging to the rare biogroup 4 were, with the exception of two serotypes (O: 92 and O: 72), serotypes previously encountered in hospital isolates (O: 73, O: 74, and 0: 75) (Penner & Hennesy, 1979a).

The third most commonly isolated member of the Proteeae was *Prot. mirabilis*, which has been reported to be the most commonly isolated species from human faeces (Rustigian & Stuart, 1945). As before, a diversity of serotypes was observed; one serotype (205/7) was observed in samples taken on weeks 9, 11, 12 and 13, suggesting persistent colonization of the calves. Unlike the two previous species, all but one of ten serotypes (O:30) were either not reported or only very rarely reported in published studies of types infecting humans in Canada (Penner & Hennessy, 1980). A similar lack of correlation is noted when the calf serotypes are compared with those from reports from Hungary (Lanyi, 1957) and the United Kingdom (de Louvois, 1969). Interestingly the widely separated hospitals all reported similar serotypes to be predominant, suggesting that certain types of *Prot.*

Table 5. *Antimicrobial sensitivity of 127 isolates of Proteeae from sites L and W*

	Ampicillin			Carbenicillin			Azlocillin			Tetracycline		
	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90	Range
Prot. mirabilis	2·0	2·0	1-2	1·0	1·0	0·5-1	2·0	4·0	2-4	64·0	128·0	8-128
Prot. vulgaris	—	—	>128	2·0	64·0	0·5->64	8·0	16·0	2-64	64·0	64·0	1->64
Prot. vulgaris BG2	—	—	>128	—	—	2-32	—	—	2-16	—	—	8-16
M. morganii	—	—	>128	1·0	1·0	0·5-1	8·0	8·0	4-16	128·0	>128·0	32->128
Prov. rettgeri	32·0	64·0	0·25-64	1·0	1·0	0·5-1	4·0	8·0	1-8	64·0	128·0	64->128
Prov. stuartii	32·0	64·0	16-64	1·0	1·0	0·5-1	16·0	32·0	8->128	—	—	>128
Prov. alcalifaciens	—	—	2	—	—	0·5	—	—	4	—	—	4

	Chlorhexidine			Cefuroxime			Ceftazidime			Trimethoprim		
	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90	Range
Prot. mirabilis	64·0	128·0	32-128	4·0	4·0	2-4	—	—	0·06	1·0	2·0	0·25-2
Prot. vulgaris	64·0	128·0	16-128	—	—	>128	0·06	0·12	0·03-0·25	2·0	8·0	0·5->64
Prot. vulgaris BG2	—	—	32	—	—	>128	—	—	0·03-0·06	—	—	0·5-1
M. morganii	32·0	64·0	32-64	64·0	64·0	32-64	0·06	0·12	0·06-0·12	1·0	2·0	1-2
Prov. rettgeri	64·0	64·0	32-64	1·0	2·0	0·12-8	0·06	0·06	0·015-0·12	1·0	2·0	0·5-2
Prov. stuartii	16·0	32·0	16-32	8·0	16·0	2-32	0·25	0·25	0·125-0·5	—	—	1
Prov. alcalifaciens	—	—	64	—	—	0·25	—	—	0·03	—	—	0·25

	Sulphonamide			Chloramphenicol			Ciprofloxacin			Gentamicin		
Prot. mirabilis	128·0	>128·0	8->128	4·0	8·0	4-8	0·06	0·06	0·03-0·06	1·0	1·0	0·5-1
Prot. vulgaris	—	—	>128	16·0	64·0	4-64	0·03	0·06	0·015-0·06	0·5	1·0	0·25-1
Prot. vulgaris BG2	—	—	>128	—	—	4	—	—	0·03	—	—	0·5
M. morganii	16·0	>128·0	16->128	32·0	64·0	16-64	0·015	0·03	0·0007-0·03	0·25	0·25	0·25-0·5
Prov. rettgeri	32·0	128·0	4-128	16·0	16·0	8-32	0·03	0·06	0·0007-0·06	1·0	2·0	0·25-4
Prov. stuartii	16·0	64·0	8->128	16·0	64·0	4-64	0·03	0·03	0·03-0·06	2·0	4·0	2-4
Prov. alcalifaciens	—	—	8	—	—	4	—	—	0·06	—	—	0·25

	Netilmicin			Cephazolin		
Prot. mirabilis	1·0	1·0	0·5-2	—	—	4
Prot. vulgaris	0·5	2·0	0·25-2	—	—	>128
Prot. vulgaris BG2	—	—	0·5	—	—	>128
M. morganii	0·5	0·5	0·25-0·5	—	—	>128
Prov. rettgeri	1·0	2·0	0·25-2	—	—	>128
Prov. stuartii	4·0	4·0	2-4	—	—	>128
Prov. alcalifaciens	—	—	0·5	—	—	<1

mirabilis persist in hospitals. It has been suggested that only certain bacteriocin types of *Prot. mirabilis* are associated with urinary-tract infection (Senior, 1979).

The finding of a range of serotypes of *Prov. stuartii* in the bedding samples was of great interest. *Prov. stuartii* has been reported to occur in calves by one group of workers in the USA, who found a single unknown O-serotype to be frequently isolated from scouring calves. Subsequent inoculation experiments suggested the strain caused neonatal diarrhoea in calves (Waldhalm, Meinershagen & Frank, 1969). Later work showed it had a synergistic effect with neonatal calf diarrhoea virus in producing scouring (Waldhalm *et al.* 1974).

For some time it was thought that faecal colonization of patients by this important nosocomial urinary-tract pathogen did not occur, but more recent work has shown it to be an important reservoir during episodes of nosocomial infection (Hawkey, 1984; Stickler, Fawcett & Chawla, *et al.* 1985). There has been interest in the natural habitat of *Prov. stuartii* and our finding of a diverse population of *Prov. stuartii* in the farm environment provides a clue as to the possible source for human gut colonization. The most frequently isolated serotypes of *Prov. stuartii* were O:25 and O:52 (two occasions each), both of these serotypes have been reported as being amongst the six most common serotypes isolated in hospitals in Canada, USA and UK (Penner *et al.* 1979*b*). The same serotypes have also been reported from a more recent episode of nosocomial infection in the USA (Hollick *et al.* 1984). There have been reports of only five different serotypes isolated from hospital patients in the UK, the second most commonly isolated serotype was O:25, which was found in the calves studied by us (Penner *et al.* 1979*b*; Epidemiological Research Laboratory, 1977). The single isolate of *Prov. alcalifaciens* was serotype O: 46, this was the second commonest serotype from a study at a Canadian children's hospital (Penner *et al.* 1979*a*).

Morganella morganii has only been reported as a rare cause of nosocomial infection, although a recent report recorded an episode of mixed infection with *Prot. mirabilis*, in which five patients were infected with *M. morganii* serotype O: 13 and three patients died (Williams *et al.* 1983). Information about *M. morganii* serotypes from hospitals is limited to a single survey (Penner & Hennessy, 1979*b*). Most strains belonged to two main O groups but considerable complexity within these groups was found. All of the serotypes reported in this paper were found previously in Canadian hospitals (Penner & Hennessy, 1979*b*), but only serotypes of the O: 1 complex occurred frequently amongst human isolates, so the calf isolates appear therefore not to belong to frequently reported human types.

The sensitivity to antimicrobials shown in Table 5 reveals the isolates to be similar in their sensitivity profiles to those reported in surveys of hospital isolates of Proteeae (Penner *et al.* 1982; Hawkey, Pedler & Turner, 1983). Although only a small number of biogroup-2 *Prot. vulgaris* were found, their MIC of 4 mg/l to chloramphenicol confirms preliminary observations made of that biogroup (Hickman *et al.* 1982). The *Prot. vulgaris* found appear to have many resistant isolates (MIC$_{90}$ 64 mg/l), which is similar to the recently reported species *Prot. penneri* (Hickman *et al.* 1982). No truly aminoglycoside-resistant Proteeae were found but some *Prov. stuartii* and *Prov. rettgeri* isolates had MIC's of 4 mg/l to gentamicin and netilmicin, which might suggest low levels of the enzyme aminoglycoside *N*-acetyl transferase (2′) (AAC 2′).

A total of 20 isolates of the 127 isolates carried plasmids, 5 of these isolates carried 2 plasmids. Six different serotypes were isolated in which plasmid-free and plasmid-containing isolates could be found with the same serotype. This finding is similar to the situation reported in *Prov. stuartii*. (Hawkey, Bennet & Hawkey, 1984; Hollick *et al.* 1984). Thus although plasmid profiles may be useful epidemiological markers, considerable care is required in using them to interpret the movement of strains.

In conclusion we have shown that bedding soiled with calf faeces and urine contains a large number of different species of Proteeae (7 of 8 described in humans) and a very wide range of different O serotypes. There appears to be a rapid 'turn-over' of many of the serotypes/species but some persisted. Comparison of the serotypes found with those reported from human infections shows a high degree of correlation. This supports the idea that Proteeae occurring in the human gut could be acquired from food animals. Gut carriage in humans has been reported as a significant reservoir for nosocomial infection in many species of Proteeae. Low-level carriage which is altered by host factors and/or antibiotic administration would then raise carriage to higher levels. An excellent example of this might be seen in *Prov. stuartii*: we have found not only quite considerable numbers of *Prov. stuartii* in bedding but also two of the most common serotypes associated with hospital infection. A recent study has questioned the source of *Prov. stuartii* causing nosocomial infection; we believe our study suggests possible sources. It is of course not possible to prove in which direction flow of organisms occurs, but it would seem reasonable to assume the main flow to be from animals to man.

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