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# Population-based laboratory surveillance for tribe Proteeae isolates in a large Canadian health region

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## ABSTRACT

The tribe Proteeae comprises the genera *Proteus*, *Morganella* and *Providencia*. Few studies have specifically investigated the epidemiology of infections caused by the Proteeae, and none has been conducted in a large non-selected population. The present study was a population-based laboratory surveillance in the Calgary Health Region (population 1.2 million), Canada during 2000–2005 that aimed to define the incidence, demographical risk-factors for acquisition and antimicrobial susceptibilities of Proteeae isolates. In total, 5047 patients were identified from whom Proteeae isolates were obtained (an annual incidence of 75.9/100 000), with females and the elderly being at highest risk. Incidence rates were 64.8, 7.7 and 3.4/100 000/year for the genera *Proteus*, *Morganella* and *Providencia*, respectively. Overall, 85% of infections were community-onset, and the overall rate of bacteraemic disease was 2.0/100 000. Compared with other species, *Proteus mirabilis* occurred at a much higher frequency, especially among females, and was less likely to be isolated from hospital-onset infections or to be part of a polymicrobial infection. Among isolates from community-onset infections, *Providencia* spp. were less likely to be from outpatients and more likely to be from nursing home residents. There were low overall rates of resistance to ciprofloxacin (4%) and gentamicin (5%), with *Prot. mirabilis* generally being the most susceptible. Members of the Proteeae were isolated frequently in both the community and hospital settings, but were infrequent causes of invasive disease. The occurrence, demographical risk-factors and microbiology of Proteeae isolates varied according to the individual species.

**Keywords** Incidence, laboratory surveillance, Proteeae, *Proteus mirabilis*, risk-factors, susceptibility

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## INTRODUCTION

The tribe Proteeae consists of three genera: *Proteus*, *Morganella* and *Providencia* [1]. These organisms cause a range of human infections of different severities, and have been associated with outbreaks of infection in hospitals and nursing homes [2–5]. While antimicrobial resistance surveys have revealed that members of the Proteeae are relatively frequent among clinical isolates, and a number of series have investigated specific subsets of infections caused by these organisms [6–10], few studies have focused on the Proteeae as a whole [11]. Kim *et al.* [11] described 132 episodes of bacteraemia involving Proteeae in a tertiary-care centre in Korea, with *Morganella morganii* being the most frequent isolate (61 cases), followed by *Proteus mirabilis* (41) and *Proteus vulgaris* (20). However, this study was performed at a single hospital and was focused solely on isolates from bacteraemia. The occurrence and determinants of all tribe Proteeae infections in a non-selected population remain to be defined. Population-based studies, in which all incident cases of a disease occurring in a defined

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geographical region are studied, minimise selection bias associated with hospital- or clinic-based studies [12,13]. The present study describes a population-based laboratory surveillance for all Proteeae isolates in a large Canadian health region, with the aim of defining their incidence, associated demographical risk-factors and microbiological features.

## MATERIALS AND METHODS

### Study population

The Calgary Health Region (CHR) provides all publicly funded healthcare services to the 1.2 million residents of the cities of Calgary and Airdre, as well as numerous adjacent surrounding communities, together covering an area in Canada of 37 000 km$^2$. Acute care is provided principally by one paediatric and three major adult hospitals, with *c.* 2000 acute-care beds and 100 000 discharges yearly. With the exception of a few small rural hospitals, all routine microbiological tests for community and hospital sites in the CHR are performed by Calgary Laboratory Services. The study included all patients from whom Proteeae isolates were obtained from specimens submitted at hospital and community-based collection sites within the CHR to Calgary Laboratory Services between 1 January 2000 and 31 December 2005. Patients were assumed to be CHR residents and were included in the analysis if they were outpatients with Alberta Personal Healthcare numbers from whom specimens were submitted to CHR-based collection sites, or if they were admitted to a CHR acute-care facility. Since laboratory data without personal identifiers were studied and individual clinical records were not reviewed, specific institutional ethics review was not required.

### Population-based surveillance

Active, population-based surveillance of all Proteeae isolates from patients in the CHR during the study was performed at Calgary Laboratory Services. Hospitals, nursing homes, physicians' offices and community collection sites were included in the surveillance. Following identification of Proteeae isolates, basic laboratory and demographical data were obtained from the regional laboratory information system (PathNet Classic v.306; Cerner, Kansas City, MO, USA). Community-onset isolates were defined as isolates submitted from community-based collection sites or nursing homes, or obtained within 2 days of admission to an acute-care facility; hospital-onset isolates were those isolated >2 days after hospital admission. In order to minimise the bias associated with repeated culturing of specimens from the same episode of clinical disease, only one culture/patient/species/year was included in the assessment of incident cases.

### Clinical laboratory testing

Proteeae isolates were obtained using standard techniques; identification and susceptibilities to antimicrobial agents were determined using the Vitek-Legacy system (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO, USA) with customised antimicrobial susceptibility testing cards, and were interpreted according to CLSI criteria [14]. At Calgary Laboratory Services, identification is performed routinely to the species level on all clinically relevant isolates, but individual speciation and antimicrobial susceptibility testing is occasionally not performed on some non-invasive isolates. In the present study, intermediately-susceptible results were grouped with and reported as resistant; when susceptibility test results were not available, rates were reported using the number actually tested as the denominator. Bacteraemia was defined by the isolation of Proteeae strains from at least one set of blood cultures. Polymicrobial episodes were those in which non-Proteeae strains were co-isolated from a single clinical specimen.

### Statistical analysis

All analyses were performed using Stata v.9.0 (Stata Corp., College Station, TX, USA). Differences in proportions among categorical data were assessed using Fisher's exact test. Medians with inter-quartile range (IQR) were used to describe asymmetrically distributed continuous variables. Incidence rates were calculated using regional demographical data as the denominator. Category-specific risks were calculated and reported as risk ratios (RRs) with exact 95% CIs, as described previously [15].

## RESULTS

During the 6-year surveillance period, 6527 Proteeae isolates were obtained from 6466 clinical specimens from 4495 CHR residents. The overall annual population incidence of Proteeae isolation was 5047 episodes, or 75.9/100 000 population; the rates for *Proteus*, *Morganella* and *Providencia* were 64.8, 7.7 and 3.4/100 000, respectively. Bacteraemia occurred in 134 patients (110 community-onset; 82%), giving an incidence rate of 2.0/100 000/year. The majority (4290 of 5047; 85%) of incident Proteeae isolates were community-onset, with 803 (19%) from emergency departments or inpatients within 2 days of admission, 970 (23%) from nursing homes, and 2517 (59%) from outpatients. Hospital-onset isolates were identified a median of 12 (IQR 6–29) days after admission to an acute-care hospital.

The median age of patients with Proteeae isolates was 69.6 (IQR 41.6–81.8) years, and 3460 (69%) were female. There was a significant relationship between age, gender and the incidence of isolation, with the rates increasing dramatically above the age of 60 years (Fig. 1). Overall, females had twice the risk of isolation of Proteeae relative to males (103.8 vs. 47.8/100 000; RR 2.17; 95% CI 2.04–2.30; p <0.0001), and this excess risk was observed across all age groups, with the exception of those aged <10 years

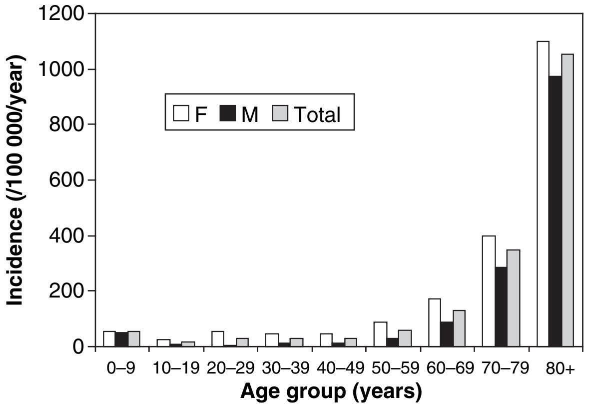

**Fig. 1.** Age- and gender-specific incidence of isolation of Proteeae in the Calgary Health Region. F, female; M, male.

(Fig. 1). Compared with males, females were significantly less likely to have hospital-onset isolates (453/3460; 13% vs. 304/1587; 19%; RR 0.68; 95% CI 0.60–0.78; p <0.0001). As with the overall isolation rate, the incidence of bacteraemia also increased significantly with increased age (Fig. 2). In contrast, no excess risk of Proteeae bacteraemia was revealed for females when compared with males (1.5 vs. 1.7/100 000; RR 0.89; 95% CI 0.59–1.32; p 0.6).

Among the entire collection of 6527 isolates, the most common specimen type was urine (5590, 85.6%), followed by wounds/soft-tissue specimens (474, 7.3%), blood (214, 3.3%), miscellaneous fluids (150, 2.3%) and respiratory tract specimens (99, 1.5%). The source of isolation correlated with the different species (p <0.001); notably, non-*mirabilis Proteus* spp. were much more likely to be isolated from wounds/soft-tissue (263/492, 53% vs. 211/6035, 3%; RR 15.29; 95% CI 13.08–17.87; p <0.0001), and were less likely to be isolated from urine (165/492, 34% vs. 5425/6035, 90%; RR 0.37; 95% CI 0.33–0.42; p <0.0001), in comparison with other species within the tribe. In total, 1905 non-Proteeae isolates were obtained as part of polymicrobial infections that included Proteeae; the most common were *Enterococcus* spp. (614, 32%), *Escherichia coli* (419, 22%), *Staphylococcus aureus* (168, 9%), and *Pseudomonas aeuruginosa* (121, 6%).

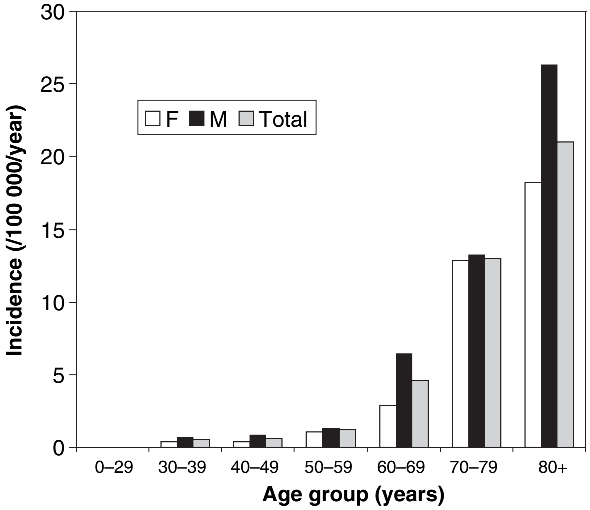

**Fig. 2.** Age- and gender-specific incidence of Proteeae causing bacteraemia in the Calgary Health Region. F, female; M, male.

In the incident cohort of 5047 cases, a number of different demographical and microbiological aspects were revealed according to the different species subgroups (Table 1). Non-*mirabilis Proteus* spp. consisted of *Prot. vulgaris* (120 cases), *Proteus penneri* (24), and un-speciated *Proteus* (260). *Providencia stuartii* was the most common incident species in that genus (149 cases), followed by *Providencia rettgeri* (72) and un-speciated *Providencia* (7). Generally, compared with other species/groups, *Prot. mirabilis* occurred at a much higher frequency, especially among females, and was less likely to be hospital-onset or to be part of a polymicrobial infection (Table 1). Among incident community-onset isolates ($n$ = 4290), *Providencia* spp. were much less likely to be isolated from outpatients (57/198, 29% vs. 2460/4092, 60%; RR 0.48; 95% CI 0.38–0.60; p <0.0001), and were much more likely to be isolated from nursing home residents (92/198, 47% vs. 878/4092, 21%; RR 2.17; 95% CI 1.84–2.54; p <0.0001), than other Proteeae.

*Prot. mirabilis* had lower in-vitro rates of resistance to most of the antimicrobial agents tested, especially β-lactams (Table 2). Overall, 4507 (96%) of 4697 Proteeae isolates were susceptible to ciprofloxacin, 4452 (95%) of 4706 to gentamicin, and 4130 (88%) of 4709 to trimethoprim–sulphamethoxazole. Although there was no significant relationship between the year of isolation and any change in trimethoprim–sulphamethoxazole resistance rates, a significantly higher rate of resistance to ciprofloxacin was observed during the final 3 years (2003–2005) of the study than during the first 3 years (2000–2002) (113/2296, 5% vs. 77/2401, 3%; p <0.01). In contrast, rates of resistance to piperacillin–tazobactam (25/1842, 1%

**Table 1.** Features of incident isolates of Proteeae according to species grouping

| Species group | Incidence/100 000 | Age (IQR) | Female | Hospital-onset | Bacteraemia[a] | Polymicrobial aetiology | Nursing home |
|---|---|---|---|---|---|---|---|
| *Proteus mirabilis* (*n* = 3903) | 58.7 | 69 (39.5–81.8) | 2845 (73%) | 498 (13%) | 98 (3%) | 827 (21%) | 758 (19%) |
| Non-*mirabilis Proteus* spp. (*n* = 404) | 6.1 | 70.7 (46.5–81.8) | 233 (58%) | 86 (21%) | 5 (1%) | 229 (57%) | 63 (16%) |
| *Morganella morganii* (*n* = 512) | 7.7 | 70.4 (47.1–81.5) | 267 (52%) | 143 (28%) | 20 (4%) | 193 (38%) | 57 (11%) |
| *Providencia* spp. (*n* = 228) | 3.4 | 72.6 (50.5–83.9) | 115 (50%) | 30 (13%) | 11 (5%) | 99 (43%) | 92 (40%) |

[a] Bacteraemia includes three cases involving *Proteus vulgaris*, one involving *Proteus penneri*, one un-speciated *Proteus*, seven *Providencia stuartii* and four *Providencia rettgeri*.
IQR, inter-quartile range.

**Table 2.** Resistance of Proteeae to common antimicrobial agents

| Species group | AMC | SXT | TZP | AMP | CFZ | CRO | CXM | CAZ | GEN | NIT | CIP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Morganella morganii*[a] (*n* = 512) | 490/491 (100%) | 68/492 (14%) | 16/114 (14%) | 491/492 (100%) | 491/492 (100%) | 1/102 (1%) | 99/100 (100%) | 31/180 (17%) | 35/492 (7%) | 363/492 (74%) | 14/490 (3%) |
| *Proteus mirabilis* (*n* = 3903) | 184/3842 (5%) | 468/3853 (12%) | 99/3835 (3%) | 623/3854 (16%) | 64/3854 (2%) | 30/3838 (1%) | 30/3834 (1%) | 30/3835 (1%) | 205/3849 (5%) | 3844/3844 (100%) | 131/3842 (3%) |
| Non-*mirabilis Proteus* spp. (*n* = 404) | 139/142 (98%) | 8/144 (6%) | 3/54 (6%) | 142/144 (99%) | 142/144 (99%) | 12/42 (29%) | 49/52 (94%) | 2/57 (4%) | 0/144 (0%) | 144/144 (100%) | 2/144 (1%) |
| *Providencia* spp.[a] (*n* = 228) | 220/221 (100%) | 35/220 (16%) | 9/37 (24%) | 219/220 (100%) | 215/221 (97%) | 1/33 (3%) | 10/33 (30%) | 6/66 (9%) | 14/221 (6%) | 211/221 (95%) | 43/221 (19%) |

AMC, amoxycillin–clavulanate; SXT, trimethoprim–sulphamethoxazole; TZP, piperacillin–tazobactam; AMP, ampicillin; CFZ, cefazolin; CRO, ceftriaxone; CXM, cefuroxime; CAZ, ceftazidime; GEN, gentamicin; NIT, nitrofurantoin; CIP, ciprofloxacin.
[a] These bacteria produce inducible AmpC β-lactamases, and the clinical relevance of susceptibity to cefazolin, ceftriaxone, cefuroxime and ceftazidime is doubtful.

vs. 102/2198, 5%; p <0.0001) and gentamicin (96/2301, 4% vs. 158/2405, 7%; p <0.001) were significantly lower in the final 3 years of the study. Cefpodoxime resistance was found in 30 (1%) of 3803 isolates of *Prot. mirabilis*; 27 of these isolates tested positive for extended-spectrum β-lactamase production according to CLSI criteria, and the remaining three isolates tested positive for AmpC production.

## DISCUSSION

This study represents the first population-based description of the Proteeae, and provides novel information on the distribution of bacteraemic and non-bacteraemic isolates. Proteeae were isolated from almost 0.1% of CHR residents/year in this study, confirming that these organisms are relatively common. Nevertheless, they rarely cause bacteraemia (2.0 cases/100 000/year). To put this in context, this rate is *c.* ten-fold lower than that reported for invasive pneumococcal (20–22/100 000) and *S. aureus* (28/100 000) disease [15–17], but is comparable to rates of *Candida* spp. bloodstream infection (2.8/100 000) [18].

The use of population-based methodology is the strength of this study. Proteeae isolates from all patients in the region were studied, including patients in hospitals, physician's offices, clinics, nursing homes and urgent-care centres, and attempts were made to exclude all isolates obtained from non-residents [13]. The fact that all isolates were included, i.e., sampling was not performed, minimised the risk of selection bias. Most studies describing infectious diseases typically involve tertiary-care centres or selected/participating laboratories, and are therefore at risk of selection bias. In addition, such studies rarely include a representative collection of samples from community-based laboratories, and may therefore fail to provide a valid picture of the overall burden or risk-factors for a particular disease in a population. For example, a previous population-based study, which demonstrated that extended-spectrum β-lactamase-producing *E. coli* strains are predominantly agents of community-onset disease, provided strong evidence against the previously held belief, supported by hospital-based studies, that this was largely a nosocomial infection [19–21].

The dramatic age-related incidence of isolation of Proteeae (Fig. 1) probably reflects, at least in part, the high rate of bacteriuria in the elderly. It should be emphasised that the rates reported in this study reflect the isolation of Proteeae, but do not necessarily indicate the presence of active infection requiring treatment. Establishing the presence of clinically significant disease with isolates associated with non-sterile sites cannot be achieved through laboratory-based surveillance alone. The major challenge with such surveillance studies is that rates of isolation of an organism will be influenced by the rate of submission of specimens for culture. As a general rule, the more severe the infection, the less this bias will be, and it is therefore probably very small for invasive or bacteraemic disease [22]. The observation of a dramatically increased rate of bacteraemia with increased age (Fig. 2) supports the hypothesis that age is a true risk-factor for development of Proteeae infections.

An advantage of the study was that it was possible to directly compare features among the different species/groups of the Proteeae in a single study. The findings that *Prot. mirabilis* was proportionally more likely to be isolated from urine and females in the community, and to exhibit increased susceptibility to antimicrobial agents, is consistent with current knowledge of the Proteeae [1]. Several studies have suggested a role for *Prov. stuartii* as an important agent in infections among nursing home residents, particularly urinary tract infections [3,4]. While the present data revealed a proportionally higher isolation rate for *Providencia* spp. among nursing home residents, it is notable that 68 (46%) of 149 isolates were obtained from acute-care hospitals and other community settings. In a review of 132 cases in a tertiary-care hospital, Kim *et al.* [11] reported that *M. morganii* was proportionally the most common cause of bacteraemia caused by Proteeae (46%), followed by *Prot. mirabilis* (31%) and *Prot. vulgaris* (15%). In contrast, the present study found that *Prot. mirabilis* was responsible for 73% of bacteraemias and that *M. morganii* caused only 15% of such cases. While the differences in the relative occurrence of these species could be caused, at least in part, by intrinsic differences between the Canadian and Korean populations, the high rate of *M. morganii* infections seen in the Korean study may reflect its hospital-based design.

The observation that *Prot. mirabilis* had much lower rates of in-vitro resistance to most of the antimicrobial agents tested than other members of the Proteeae is consistent with existing knowledge [1]. The rates of ciprofloxacin and gentamicin resistance among *Proteus* spp. were lower [6] than those in other multicentre surveillance data obtained from the MYSTIC programme. Most members of the Proteeae encode chromosomal broad-spectrum β-lactamases that confer widespread penicillin and cephalosporin resistance. Although this is not the case for *Prot. mirabilis*, several reports have documented the emerging importance of acquired extended-spectrum β-lactamase production in this species [7,10]. Extended-spectrum β-lactamase production is uncommon among *Prot. mirabilis* isolates in the Calgary region. However, the increasing annual rates of resistance to ciprofloxacin among the isolates is a concern for the future.

In conclusion, the results of this study revealed that isolation of members of the Proteeae is common in the Calgary region, and that community-based patients, females and the elderly are at highest risk, but that the Proteeae cause bacteraemic disease infrequently. The study highlighted the importance of population-based studies in accurately defining the epidemiology of an infectious disease.

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