

Isolation, identification & characterization of *Proteus penneri* - a missed rare pathogen

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Background & objectives: Indole negative *Proteus* species are invariably incorrectly identified as *P. mirabilis*, missing isolates of *Proteus penneri*. *P. penneri* is an invasive pathogen capable of causing major infectious diseases still seldom reported in individual cases. We report here the isolation, differentiation, characterization and typing of *P. penneri* from patients with different clinical infections.

Methods: Urine, pus and body fluids collected from patients in intensive care units, wards and out patients departments of a tertiary health care institute from north India were cultured. A total of 61 indole negative *Proteus* isolates were subjected to extended biochemical tests to differentiate and identify *P. penneri* from *P. mirabilis* including failure to produce ornithine decarboxylase (by 0% strains of *P. penneri* and 100% strains of *P. mirabilis*) besides *P. penneri* being uniformly salicin negative, non-utilizer of citrate but ferments sucrose and maltose. Antibigrams and Dienes phenomenon were performed to characterize and type *P. penneri* isolates besides screening for β -lactamase production.

Results: Eight isolates of *P. penneri* were identified; four from urine, three from abdominal drain-fluid and one from diabetic foot ulcer. *P. penneri* was isolated as the sole pathogen in all patients having underlying disease; post-operatively. Swarming was not seen in the first strain on primary isolation and was poor in strain-4. All eight isolates were biochemically homologous but multi-drug resistant (MDR) with resistance to 6-8 drugs (up to 12). β -lactamase production was seen in three of five isolates while Dienes phenomenon found four distinct types and discriminated strains differing in resistance even with a single drug.

Interpretation & conclusions: A few additional biochemical tests identified *P. penneri* isolates; it infected patients with underlying disease and strains were MDR and heterogenous.

Key words Dienes - indole - MDR - proteus - *Proteus penneri* - swarming

Genus *Proteus* currently has four species namely *P. mirabilis*, *P. vulgaris*, *P. penneri*, and *P. hauseri*, and three unnamed genomospecies, *Proteus* 4, 5, and 6 (single-strain *P. myxofaciens* isolated from gypsy moth)¹⁻³. *P. penneri* (formerly *P. vulgaris* biogroup 1) was recognized as a new species in 1982 but its role in clinical infection remains cryptic^{2,3}. Most infections

encountered usually are caused by *P. mirabilis* (70-90%) followed by *P. vulgaris* and rarely by *P. penneri* which is misidentified as *P. mirabilis* since both are indole negative on routine biochemical testing. *P. penneri* has ability to cause major infectious diseases and nosocomial outbreaks² and carries similar pathogenic determinants to *P. mirabilis* and *P. vulgaris*³.

P. penneri usually infects urinary tract, blood, abdominal wound, groin, neck and ankle and has been isolated mostly from urine (50%), wound and soft tissue exudates (25%), and blood cultures (15%)³⁻⁵. In a few individual case reports it has been isolated from subcutaneous abscess, urosepsis in a case with diabetes mellitus and epidural abscess, etc⁵⁻⁹. Due to lack of awareness, and inability of routine bacteriology laboratories to identify *P. penneri* isolates there are no reports on the isolation and identification of *P. penneri* from the country nor on its characterization, hence we carried out this study to isolate *P. penneri* from various clinical specimens and to characterize these isolates.

Material & Methods

Our laboratory receives several thousand samples comprising approx. 8000 urine, approx. 5000 pus and body fluids per year. Clinical specimens comprising urine, pus and body fluids received from ICUs, wards and out patients departments of Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow, a tertiary health care center in north India were collected and cultured routinely for bacterial isolations, and identification in the department of microbiology over a decade (1997-2007). The clinical specimens were first screened microscopically by Gram's stain, then cultured on blood agar (aerobically and anaerobically), MacConkey agar and Robertson cooked meat broth for 48 h at 37°C in 5-10 per cent CO₂. Urine specimen were examined microscopically as wet mount and then its semiquantitative cultures were done on cystine lactose electrolyte deficient (CLEED) agar medium (Hi-Media Laboratories, India) and sheep blood agar media and incubated at 37°C for 18-24 h.

All the isolates that grew from urine (10⁵ cfu/ml), pus and body fluids were at first subjected to standard routine biochemical tests and provisionally diagnosed as *Proteus* species based on the findings of non-lactose fermenting colonies with (or without) swarming on blood agar plate, characteristic fishy smell, Gram negative pleomorphic bacilli with active motility and that reduced nitrate, produced catalase but not oxidase, fermented glucose (with acid and gas), sucrose and mannitol but not lactose, usually did not utilize citrate but hydrolyzed urea and produced phenyl pyruvic acid, were identified as *Proteus* species^{3,5,10}.

Indole negative *Proteus* species as identified above were then picked up whenever possible during the study period stocked and maintained in nutrient agar stabs and later identified by putting extended biochemical

tests as laid down in the manual for the identification and differentiation of *P. penneri* isolates¹⁰. The extended, simple differentiating biochemical tests are shown in the Table, among these additional production of ornithine decarboxylase and fermentation of salicin and maltose were included in the present study. The most reliable single test and the absolute biochemical criteria used was failure of production of the enzyme ornithine decarboxylase (after 5-7 days of incubation) by *P. penneri* (0%) in contrast 100 per cent strains of *P. mirabilis* which produces this enzyme¹⁰. Further, *P. penneri* isolates are non-fermenter of salicin and non-utilizer of citrate but acidify sucrose and maltose¹⁰. Antibiotic sensitivity tests were done in all eight isolates soon after their isolation against 18 antimicrobial drugs viz., ampicillin, ampicillin plus clavulanic acid, amikacin, gentamicin, cotrimoxazole, ciprofloxacin, cefuroxime, ceftazidime, chloramphenicol, cefotaxime, nalidixic acid, norfloxacin, tetracycline, cefodroxil, meropenem, ceftriaxone, cephalixin and ticarcillin by modified Stokes' disc diffusion method¹¹. Additionally, Dienes phenomenon was tested later on in five isolates (as three isolates 4U, 5U and 8P were lost to subcultures) using freshly prepared blood agar culture plates as per standard procedures¹² for further typing and comparison of *P. penneri* isolates. Screening for β-lactamase production was tested by nitrocefin method using standard procedure¹³. Briefly, a disc of nitrocefin (Becton Dickinson, USA) made wet with sterile distilled water was put over the colonies of an overnight culture plate and examined after 30 min for development of pink colour in the disc if β-lactamase was produced while no colour was developed in non-producers of this enzyme. β-lactamase production was done on the same five isolates of *P. penneri* which were tested for Dienes phenomenon. The results of antibiograms of isolates and Dienes phenomenon were compared and utilized for the characterization and typing of *P. penneri* into strains.

Results & Discussion

Among 61 indole negative *Proteus* species subjected to detailed biochemical analysis, eight (13.1%) isolates of *P. penneri* were identified (Table). Majority of the *P. penneri* infected patients were males (5 of the 8 cases) and belonged to a wide age range (3-73 yr). In all the eight cases, *P. penneri* was the sole pathogen as it was isolated in pure culture and all cases had some underlying disease (Table). Five of the eight isolates of *P. penneri* showed β-haemolysis on sheep blood agar. Biochemically the strains were quite homologous

except a few reactions, e.g. one strain was citrate positive and H₂S negative. *P. penneri* are known to be non-producer of the enzyme ornithine decarboxylase and are uniformly salicin negative but acidify maltose. These characters were strictly adhered to and were also seen among all the isolates in this study.

Antibiogram were different for different isolates and all were multi-drug resistant (MDR) with resistance to 6-8 drugs and even to 12 drugs in one isolate (strain 5). Strain No. 1 and 2 had quite similar antibiogram except for being sensitive to chloramphenicol. Strain-3 was also nearly similar to first two except being sensitive to co-trimoxazole, nalidixic acid and norfloxacin. Among the β -lactams, ampicillin proved to be the least efficient and all isolates were resistant to ampicillin,

first and second-generation cephalosporins (cephalexin and cefuroxime). Third generation cephalosporins (ceftazidime, cefotaxime and ceftriaxone) showed better although heterogeneous, *in vitro* sensitivity. β -lactamase production was seen in three of the five isolates tested. The antimicrobial drug sensitivity tests done in the present study and by other authors too were according to modified Stokes' disc diffusion method, being prevalent that time while CLSI guidelines came much later in the year 2005-06 and were adopted gradually.

Swarming was not seen in the first strain-1 on primary isolation while growth and swarming was poor in the strain-4. On the basis of Dienes phenomenon (done on the 5 isolates) it was observed that four

Table. Clinical characteristics, predisposing factors and biochemical identification of eight isolates of *P. penneri*

Isolate No. (Strain no.)	Ageyr/ Sex	Sample	Diagnosis/ Predisposing-factors	Swarming	Haemo-lysis	Salicin	Citrate	Ornithine decarboxylase	Sucrose	Maltose	H ₂ S production
1U (1)	3/ M	Urine	PUV & recurrent cystitis	+ **	+	-	+	-	+	+	-
2U (2)	47/ M	Urine	Genito-urinary TB	+	+	-	-	-	+	+	+
3U (3)	38/ F	Urine	VVF & stricture urethra (Post-op)	+	-	-	-	-	+	+	+
4P	61/F	Pus	Diabetic foot	+	-	-	-	-	+	+	+
5U	59/ M	Urine	Acute On CRF	+	+	-	-	-	±	±	+
6P (4)	73/ M	Drain fluid	Carcinoma rectum (Post-op)	++	-	-	-	-	+	+	+
7P (5)	13/ M	Drain fluid	Juvenile polyposis coli (Post-op)	+	+	-	-	-	+	+	+
8P	46/ F	Drain fluid	Cholelithiasis (Post-op)	+	+	-	±	-	+	+	+

**No swarming seen on primary isolation; *poor growth and swarming; PUV, post urethral valve (and this patient underwent cystoscopy); VVF, vesico-vaginal fistula; CRF, chronic renal failure; U, urine; P, pus

strains (strains-1, 2, 3, and 5) were distinct from one another as swarming edges did not merge with one another but showed demarcation line of non-identity while strain-4 could not be typed due to its poor growth and swarming. Typing by Dienes phenomenon was more discriminative since strains-1 and 2 had identical antibiogram except in sensitivity to single drug namely chloramphenicol but were distinct Dienes types. Three isolates were lost due to long period of study.

Most bacteriologists are largely unaware of species *P. penneri* and even if aware no attempts have been made to find its clinical significance. Hence, there is a scarcity of large series^{14,15} reports on the isolation of *P. penneri* from infected patients. Another reason is that *P. penneri* was recognized as a new species in 1982 only. In the present study, among indole negative *Proteus* species isolated, only 13.1 per cent (8/61) isolates were *P. penneri*. In a study from Taiwan among all positive urine cultures from hospitalized patients with urinary tract infection/urinary calculi an incidence of 2.1 per cent for *P. penneri* was reported¹⁴. Another study from Korea¹⁵ focusing on bacteraemia due to tribe *Proteaceae* isolated 132 strains, but only two were *P. penneri*. In the present study, *P. penneri* was isolated from post-operative patient's urine, drain fluid or pus and who had some underlying disease besides diabetic foot ulcer, which was found to be novel presentation.

Since *P. penneri* may be non-swarming on first isolation, extra vigilance is required. Infections due to *P. penneri* (and also *P. mirabilis* and *P. vulgaris*) could be more pronounced, persistent and difficult to eradicate owing to being an invader pathogen with several virulence factors¹⁶ besides swarming and haemolysin and production of proteolytic enzymes. The major mechanism of antimicrobial drug resistance is determined chromosomally^{3,17} with hyperproduction of the chromosomally encoded beta-lactamase, occasionally due to plasmids. Besides *P. penneri* strains are naturally resistant¹⁸ to penicillin G, amoxicillin, cephalosporins (*i.e.* cefaclor, cefazoline, cefuroxime and cefdinir), oxacillin, most of macrolides but are naturally sensitive to aminoglycosides, carbapenems, aztreonam, quinolones, sulphamethoxazole and cotrimoxazole. Most of isolates in the present study were MDR including resistance to amoxy-clavulanic acid combination (except one isolate). In one study, *P. penneri* was found to be more resistant to the penicillins and cephalosporins than *P. mirabilis* and mostly in patients with urogenital infections¹⁸. This further emphasizes the need for species level identification of *P. penneri*.

In the present study, beta-lactamase production was seen in three of the five isolates; however, all eight *P. penneri* isolates were uniformly sensitive or moderately sensitive to ceftazidime, cefadroxyl and meropenem and could be treated successfully except the first case who had post-urethral valve.

Typing by antibiogram found *P. penneri* to be highly heterogeneous MDR strains and hence was of limited value. Moreover, strain differentiation by antibiogram and Dienes phenomenon did not show concordance. Typing by Dienes phenomenon was more discriminative as it differentiated strains even if it differed in resistance with a single antibiotic, *e.g.* strains-1 and 2 and is still employed¹⁹.

Hence efforts should be made to identify *P. penneri* infections by using a few additional biochemical tests in a large series studies by ribotyping and pulse field gel electrophoresis (PFGE) in order to find its incidence, predisposing host factors and the clinical significance of this missed bacterium. Further, patients with underlying diseases going for abdominal or urogenital surgeries should be examined carefully for *P. penneri* infections because of their MDR nature with potential to spread drug resistant plasmids nosocomially.

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