

Original Research Article

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A Tale of Two Novel *Proteus* Species-*Proteus hauseri* and *Proteus penneri*

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ABSTRACT

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The present study reporting two different cases of two new *Proteus* species-*Proteus hauseri* and *Proteus penneri* isolated from two different patients. One case reports a one month old male child with history of diarrhoea since six days. His stool sample was send for microbiological examination and the second case is of Ante natal care patient with urinary tract infection whose urine was send for microbiological examination. Both of these isolates showed Non lactose fermenting colonies. There was no swarming on Blood agar. Biochemical reactions revealed *Proteus* and both of these isolates were sent for further identification to National Chemical Laboratory, Pune for further identification. First isolate was identified as *Proteus hauseri* and second one as *Proteus penneri*. Both the patients were treated successfully with meropenem and were discharged few days later. As per our knowledge, this is the third case of *Proteus hauseri* and few cases of *Proteus penneri* have been reported so far. So, hereby we are presenting with two cases of novel species of *Proteus*. Both of these species are very rare and as there was no swarming on blood agar these isolates may be misdiagnosed.

Introduction

The genus *Proteus* along with genus *Providencia* and *Morganella* belongs to the tribe *Proteae* of the family *Enterobacteriaceae*. (Nita Pal *et al.*, 2014) Hauser first noted the swarming nature of the organisms and divided the strains into the two species based on the speed of their ability to liquify gelatin: *P. vulgaris* liquefies gelatin “rapidly,” and *P. mirabilis* does so “more slowly”. (Hauser, 1892) Hauser also described “*Proteus zenkeri*,” which neither swarmed nor liquefied gelatin, but he rescinded this particular finding 7 years later .The genus

*Proteus* currently consists of four named species (*P.mirabilis*, *P.penneri*, *P.vulgaris*, *P.myxofaciens*) and four unnamed genomospecies Genomospecies 3 was named *Proteus hauseri* to honor Gustav Hauser, the German microbiologist, who first described the genus. (Caroline Mohr O'Hara *et al.*, 2000) In 1982, *P. vulgaris* biogroup 1 (genomospecies 1) was named *Proteus penneri* and was distinguished by its negative reactions for indole production, salicin fermentation and aesculin hydrolysis. The remaining two biogroups were both positive for indole pro- duction. However,

biogroup 2 (genomospecies 2) was positive for salicin and aesculin and biogroup 3 was negative for salicin and aesculin. (Caroline Mohr O'Hara *et al.*, 2000) *Proteae* are widespread in the environment and make up part of the normal flora of the human gastrointestinal tract.<sup>1</sup> *Proteus* species are among the commonly implicated pathogens in hospital as well as community acquired infections. (Patrick Kwame Feglo *et al.*, 2010) This pathogen has a diverse mode of transmission, and hence can cause infection in different anatomical sites of the body. Some of the incriminating sources of transmission are soil, contaminated water, food, equipments, intravenous solutions, the hands of patients and healthcare personnel *Proteus* ranks third as the cause of these infections, particularly in hospital-acquired cases. *P. mirabilis* accounts for approximately 3% of nosocomial infections in the United States (Centers for Disease Control and Prevention, 1996) and is commonly isolated in clinical microbiology laboratories. Both *P. mirabilis* and *P. vulgaris* are widely distributed in the environment with reservoirs in soil, water, sewage and feces and have been isolated from the intestinal tract of mammals, birds and reptiles. *Proteus penneri* has been isolated from a number of diverse clinical sites, including abdominal wounds, urine, bladder calculi, epidural ulcers, bronchoalveolar lavage fluid, stool and infected conjunctiva. (Krajden *et al.*, 1987) Indole negative *Proteus* species are invariably incorrectly identified as *Proteus mirabilis*, often missing out isolates of *Proteus penneri*. The urease enzyme of *P. penneri* is also believed to be a leading cause of kidney stone formation. (Pearson *et al.*, 2008) Very few case reports of *Proteus penneri* have been reported so far. And only one case report of *Proteus hauseri* by Caroline O' Hara has been reported. We are hereby reporting case reports of these

*Proteus* species.

## Materials and Methods

Clinical profile of patient was recorded. Samples were subjected to microscopy, culture and antibiotic susceptibility testing according to Kirby Bauer disk diffusion method using disk of Hi- Media laboratories, Mumbai.

**Case 1-** A month old boy was brought by his parents with complaints of passing loose stools 8-10 times a day since 6 days. He had fever, persistent cry and was unable to accept feeds. There was no neck stiffness and other symptoms of meningitis. The baby was full term, born by caesarean section. His stool sample was sent to microbiology department for Hanging drop, wet mount and culture.

Grossly, the stool was liquid, mucoid, greenish, non blood stained and was adherent to the container.

Microscopically,

Hanging drop- evidence of motile bacilli.

Wet mount revealed abundant pus cells, few RBC's, cyst of *Entamoeba histolytica* and motile bacilli.

Culture was done on MacConkey's agar, TCBS and blood agar.

MacConkey's Agar showed, Non lactose fermenting, circular, smooth, irregular margin, 3-4mm colonies, catalase positive and oxidase negative colonies. Blood agar showed 3-4mm, circular, smooth, irregular margin, non haemolytic colonies without swarming.

TCBS showed greenish, 2-3mm, irregular margin colonies.

Colonies from MacConkey's agar were subjected to biochemical reactions and Antibiotic susceptibility testing.

**Case2-** This case reports a 22 years female, with 6 months amenorrhoea came with complaints of fever, increase in frequency of micturation, pain and burning during micturation since 5 days. Her clean caught midstream urine sample was collected in morning and was send to microbiology department for microscopy and culture.

Grossly, urine was turbid, yellow, non odorous.

Microscopically on wet mount there were 5-6 pus cells/high power field and motile bacilli.

Urine was cultured on CLED agar. After overnight incubation, 3-4mm, yellow, circular, irregular margin colonies were seen that were catalase positive and oxidase negative. These colonies were subjected to biochemical reactions and Antibiotic susceptibility testing.

Repeat samples of both the patients were taken to rule out contamination.

The antibiotic susceptibility testing by Kirby Bauer disk diffusion method is as follows (Clinical and Laboratory standards Institute, 2014)

Case-1 Amikacin (9mm), ciprofloxacin (12mm), ceftriaxone (8mm), ceftazidime (11mm), ceftazidime plus clavulanic acid (14mm), meropenem (10mm), colistin (22mm).

Case-2 Amikacin (6mm), ciprofloxacin (8mm), ceftriaxone (6mm), ceftazidime (12mm), ceftazidime plus clavulanic acid (14mm), meropenem (25mm).

Both the patients were treated with meropenem. The first patient was discharged after 10 days and second after seven days.

The biochemical reactions revealed *Proteus* species but the colonies on Blood agar were non swarming. So, both the strains were sent to National Chemical Laboratory, Pune for further identification.

The first isolate was confirmed as *Proteus hauseri* and second as *Proteus penneri* by 16sRNA PCR.

To prove the pathogenicity of these two isolates, intradermal inoculation was done in mice and after taking all necessary precautions. The site of intradermal inoculation was marked. And after 48 hours, a blister was present at injected site. It was around 1cm. Blister fluid was taken for culture and it also revealed same microorganisms.

## Discussion

**Case-1:** In a study by Müller, *P. mirabilis* and *P. penneri* were isolated significantly more often from stools of patients with diarrheal disease than from healthy patients, leading him to speculate that these species may play a role in some diarrheal disease (Müller, 1986). Their true role, however, remains unsubstantiated. However, *P. hauseri* from stool has not been reported. Our isolate is from stool sample. *Proteus* generally shows swarming on blood agar. Only one article has been published in year 2000, by Caroline O Hara in which out of 52 isolates only two were *P. hauseri*. They have not mentioned whether swarming was present or not. Our isolate did not show swarming. *Proteus hauseri* can be distinguished from *P. mirabilis*, *P. penneri* and *P. myxofaciens* as it is positive for

Indole production and all other three are negative. This is the main distinguishing feature by which *P. hauseri* can be distinguished. Anti- microbial susceptibility patterns, of O'Hara being susceptible to amikacin, ceftazidime, ciprofloxacin, imipenem, and tri- methoprim

sulfamethoxazole. They were resistant to tetracycline. Our isolate was only sensitive to meropenem. The patient was treated successfully with meropenem and was discharged after ten days. Due to non swarming nature, many isolates of *P.hauseri* can be missed.

Biochemical Tests of 2 Cases are as follows<sup>9</sup>

Tests	Case1	Case2
Indole	Positive	Negative
Methyl Red	Positive	Positive
Voges Proskauer	Negative	Negative
Citrate(Simmons)	Was utilized	Was utilised
Urease	Was hydrolysed	Was hydrolysed
Triple sugar iron	Acid/acid with H <sub>2</sub> S	Acid/acid with H <sub>2</sub> S
ONPG	Negative	Negative
Nitrate	Was reduced to nitrite	Was reduced to nitrite
Bile esculin	Not hydrolysed	Not hydrolysed
Phenylalanine deaminase	Positive	Positive
Ornithine decarboxylase	Negative	Negative
Arginine dihydrolase	Negative	Negative
Lysine decarboxylase	Negative	Negative
DNase	Negative	Negative

**Figure.1** Showing Non Swarming Growth of *Proteus hauseri* on Blood Agar



Figure.2 Showing Biochemical Reactions of *Proteus hauseri*



**Case-2:** *P. penneri* was absent in samples obtained from < 1, 50 - 59 and 70 - 79 years age groups. (Jitendra Kumar Pandey *et al.*, 2013) Very few case reports are there with *P. penneri* infection between 20-30 years of age. Our patient was 27 years old. *P. mirabilis* was the only *Proteus* species encountered in urine samples and this supported the finding that *P. vulgaris* and *P. penneri* infections of the urinary tract are rare. (Chung *et al.*, 1999) *P. penneri* is mostly isolated from wound swab, and next from urine samples. *Proteus* spp. (*P. mirabilis*, *P. vulgaris*, and *P. penneri*) are important pathogens of the urinary tract and the primary infectious agents in patients with long-term indwelling urinary catheters. (Jacobsen *et al.*, 2008) Our isolate is from urine sample from a non catheterized patient. *P. penneri* shows swarming on blood agar. However non swarming strains mainly on first isolation is seen in many cases (Janak Kishore, 2012). Multiple drug resistance in *P. penneri* is common. *P. penneri* was the most resistant among the recovered species. Our isolate was sensitive only to meropenem and resistant to all baseline antibiotics.

In conclusion, *P. hauseri* and *P. penneri* may not show swarming and hence could be

missed. As they are most resistant *Proteus* species prompt diagnosis and early treatment is required to save the patient.

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