Antibiotic Sensitivity against *Proteus Mirabilis* Isolated from Pus Samples in Tertiary Care Hospital of Quetta, Pakistan

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Abstract

Proteus is an important microorganism that have been isolated from wound infections causing severe complications. This study was aimed to isolate and determine the antibiotic sensitivity profile against isolates. A total of 50 wound and pus samples were collected from patients admitted in BMC Hospital, Quetta and processed in Pathobiology Laboratory of Center for Advance Studies of Vaccinology and Biotechnology (CASVAB) University of Baluchistan. All the samples were inoculated onto Brain Heart infusion (BHI), MacConkey agar, Cysteine-Lactose-Electrolyte-Deficient (CLED) and Blood agar and incubated at 37 °C, overnight. The suspected colonies were identified by gram stain and different biochemical tests and also confirmed by local made Quick Test Strip 12 (QTS 12). Among the total 50 samples 8 were found positive for *Proteus mirabilis*. Antimicrobial susceptibility of *Proteus mirabilis* against different antibiotic was evaluated by Kirby-Bauer Method. Four different antibiotics were tested and ampicillin was found highly susceptible (75%), followed by Gentamicin (50%), Cefotaxime (40%) and ciprofloxacin (40%). This study shows the incidence of *Proteus mirabilis* in wound infection and best possible antimicrobial therapies against its infection.

Keywords: *Proteus*, Wounds, Kirby-Bauer, Hospitals, Quetta

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INTRODUCTION

Genus *Proteus* is a member of family Enterobacteriaceae. It is a Gram-negative rod in gram staining. Many species are member of genus *Proteus* i.e. *Proteus mirabilis*, *Proteus hauseri*, *Proteus penneri* and *Proteus vulgaris*. This genus is usually Saprophytic in nature but the Many Member of this genus are opportunistic pathogens ie *Proteus mirabilis*, *Proteus penneri* and *Proteus vulgaris*. These are involved in septic diseases, nosocomial and genito urinary tract infections (1). *Proteus* is part of normal flora of Gastrointestinal tract. It is wide spread in nature and is 3rd major causative agent of nosocomial (hospital acquired infections) (2,3).

Usually it is not a pathogenic organism by it self but play role in secondary bacterial infection and create problems when reaches in urinary tract or other uncommon places. *P. mirabilis* is involved in many urinary tract problem in thousands of patients along with other opportunistic bacteria (4).

It can be identified on blood agar by production of concentric bracelets due to its crawling association and indole production is another distinguishing character of *P. mirabilis* from other proteus species (5).

Proteus mirabilis is Gram-negative, facultative anaerobic bacilli. It is characteristically urease positive and shows swarming motility movement on blood agar. About 90% of all

Proteus contaminations in humans are due to *Proteus mirabilis*. In many other infection the presence of *Proteus spp.* is notable such as ear, nose and throat (ENT) infection, respiratory tract, skin burns and gastroenteritis problems (1). Number of biochemical tests like indole, citrate, Motility, Oxidase, phenylalanine deaminase etc can be used for identification of *Proteus* species (6). A focused study on *Proteus mirabilis* causing nosocomial infection with emphasis on subclinical type is lacking in Quetta. It is therefore important to assess the effect of the *Proteus mirabilis* in case of pus infectious in Quetta and is necessary to estimate loss attributed to the disease. This study aims to isolate *Proteus spp.* from Pus infection samples and to characterize it biochemically and evaluate antibiotic sensitivity profile.

MATERIAL AND METHODS

Study area and Population

The study was performed at (post graduate laboratory) Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB) Brewery Road during June-Dec, 2016. A verbal consent was taken from 50 indoor patients that were included in this study.

Sample collection and media inoculation

A total of 50 pus samples were collected aseptically from wounds of indoor patients using swabs. All the samples streaked on Brain heart infusion (Oxoid, United Kingdom) agar, Blood agar base (Oxoid, United Kingdom) and CLED agar (Oxoid, United Kingdom).

The specimen were inoculated initially on BHI broth and on next day shifted on Blood agar and Mac-Conkey agar and and Cysteine-Lactose-Electrolyte-Deficient (CLED) agar for identification of *proteus* characters and incubated at 37°C for 24 hrs. Different characteristics features of colonies were also noted such as, shape, size, color, hemolytic nature and pigmentation pattern.

Biochemical Characterization

Suspected *Proteus* colonies were triple cloned and gram staining of the purified colonies was performed. Colonies showing swarming growth on blood agar were subjected to different Biochemical test like Indole, Methyl Red, Vogas prausker, Citrate utilization, Triple sugar iron, nitrate reduction, H₂S gas production, Oxidase, Catalase and urease production test (7).

Quick Test Strip 12 (QTS 12)

For the identification of few oxidase positive and most oxidase negative i.e Enterobacterae the test is successful (8). QTS 12 before inoculation is shown in (Fig. 1)

Antibiotic Sensitivity Test (AST)

Different antibiotic discs were used against *P. mirabilis* isolates following Kirby-Bauer disk diffusion method. The plates were then incubated at 37°C for 24 hrs and the zone of inhibition was measured in accordance with Clinical and Laboratory Standards Institute (CLSI). Commercially available Mueller-Hinton agar (Oxoid, UK) media and antimicrobial discs (Oxoid, UK) were used for the assay. The inoculum was prepared and matched with 0.5 Mc Farland standard as proposed by (7).

RESULTS

Out of 50 clinical samples total of 8 isolates were confirmed as *Proteus mirabilis* by different biochemical tests as shown in (Table I).

Cultural Characteristics

The isolates were grown on different media. *Proteus* gave swarming growth on Blood agar (Fig. 3) and non-lactose fermented colorless pale colonies on Mac Conkey agar were produced.

Microscopy and Biochemical Characteristics

Microscopically the isolates were gram negative pink rods when stained with gram stain. All the 8 purified triple cloned isolates of *Proteus mirabilis* were subjected to different test like Indole, Methyl red, Vogas prausker, Citrate utilization, H₂S gas production, Triple sugar iron, nitrate reduction, Oxidase, Catalase, urease reactions and results of all biochemical test are illustrated in (Table II). Biochemical test results of QTS 12 are given in Fig 2 and Table III.

Antibiotic Sensitivity of *Proteus* Isolates

All isolates (n=8) were tested for sensitivity to four different antibiotics. *Proteus mirabilis* showed high sensitivity against ampicillin (23 mm), followed by Gentamicin (18 mm), Ciprofloxacin (17 mm), and Ceftazidime (18 mm). Detail is given in Table II.

Table I. Different biochemical tests performed for the isolation and characterization of *Proteus mirabilis* spp from pus infection in Quetta, Pakistan.

Sr #	Basic Characteristics	Result
1	Catalase	Positive
2	Citrate utilization	Positive
3	Gelatin Hydrolysis	Positive
4	Indole production	Negative
5	Methyl Red	Positive
6	Nitrate Reduction	Positive
7	Oxidase	Negative
8	Urease	Positive
9	Voges Proskauer	Negative
10	Motility	Positive

Table 2. Inhibitory zones against *Proteus mirabilis* of different antibiotics

Bacterial strain	Zone of inhibition (mm)			
	Ampicillin	Gentamycin	Ceftazidime	Ciprofloxacin
<i>Proteus mirabilis</i>	23	18	18	17

Fig. 1. Quick test Strip 12 before inoculation

Fig. 2. Quick test Strip 12 after inoculation and incubation

DISCUSSION

Nosocomial infections are serious problems acquired by patients during their visits to health care centers. Many predominant bacterial infection are associated with *Staph aureus*, *E coli* and *Pseudomonas aurogenosa*, *proteus spp*, etc. This study shows the occurrence of *proteus mirabilis* in wound infection. This organism is also present more frequently in urinary tract infection. Genus *Proteus* as a members of the Enterobacteriaceae occur widely in human, animals and in the environment and can be readily recovered from sewage, soil

Table III. Different Biochemical and sugar tests performed for the characterization of isolates.

Sr #	Test	Reaction status	Colour shown
1	Indole	-	yellow
2	Glucose	+	brown
3	ADH	+	Royal blue
4	Urease	+	Pink
5	Gel liquefaction	+	black
6	ONPG	+	yellow
7	Citrate	+	Blue
8	LDC	+	green
9	ODC	+	Royal blue
10	Hydrogen sulphide	+	black
11	Sucrose	+	yellow
12	Voges proskaur	+	Red ring

Fig 3. Swarming of *Proteus mirabilis* on blood agar plate

and vegetables etc (9). It is important to minimize its occurrence in infections that may further complicate the cases. *Proteus mirabilis* (n=8) were isolated in present study from wound infection. Different biochemical tests were applied for identification. Several other researcher also isolated the *proteus spp* from wound infection using such type of traditional techniques (10-13).

Despite the literature cited no such work has been reported in the Quetta area. As *Proteus* species are found in multiple environmental habitats including long term care facilities and hospitals and important clinical infections are produced due to this organism. It is important to have the surveillance and estimation program in order to plan strategy to combat such infections.

Antibiotic sensitivity and resistivity profile was constructed using Kirby- Bauer disk diffusion method against *P. mirabilis* and data regarding measurement of zone of inhibition was collected according to Clinical and Laboratory Standards Institute (CLSI) protocol. AST was performed on Muller Hinton agar (Oxoid, UK) by using commercially available antimicrobial discs (14).

Highest inhibitory zone was shown by Ceftazidime (23 mm) followed by Gentamycin (18 mm) and Ciprofloxacin (17 mm)

which confirms that these antibiotics can be used for clinical purposes for the treatment and management of wounds.

According to this study Gentamycin and Fluroquinolones were found as sensitive and effective drugs in wound infection. In addition this study also gives the common profile of infected wounds regarding bacterial infection and give a guideline for treatment of wound infection due to proteus. Review of literature also prove gentamycin as sensitive drug against proteus infections (15).

CONCLUSION

These findings highlights that Genatmycin and Quinolones can be used in the treatment of wound infections complicated with proteus organisms. This study serves as base line and may be helpful for the clinicians while managing the treatment against wound infections.

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