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Characterization of *Proteus mirabilis* Isolated from Patient Wounds at Bolan Medical Complex Hospital, Quetta

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Abstract

Background: Wound infection is the cause of mortality and morbidity on a global scale. Microorganisms infecting wounds can multiply and colonize in the wound, resulting in host tissue damage.
Objectives: The present study was conducted to identify *Proteus mirabilis* in wounds of patients in Quetta district.
Methods: This study was conducted from June 2017 to June 2018 at the Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta. Overall, 480 different wound samples were collected from patients admitted to Bolan Medical Complex Hospital, Quetta. *Proteus mirabilis* was isolated using differential and selective media and characterized by biochemical tests (catalase, oxidase, IMViC, and sugar fermentation), antimicrobial susceptibility tests, and PCR.
Results: There were 64 (13.3%) samples positive and 416 (86.6%) samples negative for *P. mirabilis*. The results showed that wounds infected with *P. mirabilis* were more common in male patients (n = 40; 8.3%) than in female patients (n = 24, 5%). The age distribution showed that the infection of wounds with *P. mirabilis* was the highest in 16 - 30-year-old group (n = 32; 6.70%), followed by the age groups of 5 - 15 (n = 24; 5%) and 30 - 50 years (n = 8; 1.60%). Diabetic (n = 24; 5%) and surgical (n = 24; 5%) wounds were more affected by *P. mirabilis* than burn wounds (n = 16; 3.30%). *Proteus mirabilis* was sensitive to gentamicin (n = 50; 78%) and amikacin (n = 53; 82.8%) but resistant to penicillin G (n = 58; 90%), ampicillin (n = 56; 87.5%), amoxicillin (n = 60; 93.7%), cefuroxime (n = 61; 95.3%), ceftriaxone (n = 57; 89%), ceftazidime (n = 59; 92.1%), imipenem (n = 62; 96.8%), ciprofloxacin (n = 55; 85.9%), and tetracycline (n = 59; 92%). The PCR-based identification of *P. mirabilis* showed clear bands of 533 bp of the ureC1 gene.
Conclusions: The pathogenesis of *P. mirabilis* in wound infection and its antimicrobial sensitivity are major problems worldwide. The use of aminoglycosides such as gentamycin and amikacin is effective against *P. mirabilis* and can help prevent the spread of infection and reduce the cost of treatment. The PCR technique is one of the sensitive, timesaving, specific, and cost-effective ways for the identification of the pathogenic genes of *P. mirabilis*.

Keywords: *Proteus mirabilis*, PCR, Antibiotic, Wound Infection

1. Background

Proteus species belong to the Enterobacteriaceae family (1). There are several species of *Proteus* including *Proteus mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri*, *P. myxofaciens* (2, 3), *P. alimentorum*, *P. cibarius*, *P. columbae*, *P. inconstans*, *P. morganii*, *P. terrae*, and *P. rettgeri* (4-7). *Proteus mirabilis* is a Gram-negative, rod-shaped, facultative, anaerobic, non-capsulated, non-spore forming, and motile bacterium (8). It is mostly found in natural environments and responsible for the infection of the pulmonary system, burns, skin, eyes, ears, nose, and the urinary tract, as well as gastroenteritis (9, 10). *Proteus mirabilis* is the third most common cause of nosocomial infections accounting for 90% of all *Proteus* infections (11).

A major problem in wound infections is the ever-rising antimicrobial resistance in *P. mirabilis* (12-14). *Proteus mirabilis* may become resistant to β-lactams upon the acquisition of heterologous β-lactamase genes (15). A multidrug-resistant *P. mirabilis* clone with chromosomal AmpC-type beta-lactamase was reported in the literature (16). Thereby, *P. mirabilis* may be resistant to broad-spectrum β-lactams including penicillins and cephalosporins (17).

The virulence of *Proteus* spp. is dependent on the several virulent factors that are regulated by several genes encoded in operons (18). *Proteus mirabilis* utilizes the urease

enzyme that plays an important role in the pathogenesis of kidney and bladder stone formation. Some genes such as ureA, ureB, ureC, ureD, ureE, ureF, ureG, and ureR on the ure operon are responsible for the production of the urease enzyme among which, ureC is a major contributor (19)

2. Objectives

The present study was designed to evaluate the different aspects of *P. mirabilis* and its antibiotic susceptibility pattern.

3. Methods

3.1. Collection of Wound Samples

The study was conducted from June 2017 to June 2018 at the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta.

Overall, 480 wound samples (160 burn, 160 surgical, and 160 diabetic wounds) were collected from patients admitted to Bolan Medical Complex (BMC) Hospital. The samples were collected using pre-sterile pre-labeled cotton swabs and taken to the laboratory in cold chain condition. Data regarding the patients' history, age, gender, and wound type were collected using predesigned checklists.

3.2. Sample Processing

The collected samples were streaked on differential (MacConkey agar, Oxoid) and selective (Proteeae isolation medium agar, Oxoid) media and incubated at 37°C for 24 h. All isolates were triply cloned to get fresh colonies for Gram staining, biochemical tests (Indole, Methyl red, Voges-Proskauer, Simmon Citrate, Oxidase, and Catalase), and PCR.

3.3. Antimicrobial Susceptibility Test

Antimicrobial susceptibility was tested by the disc diffusion method according to the clinical and laboratory standards institute guidelines (CLSI guidelines, 2006). A suspension of bacterial cells (0.5 McFarland) was prepared and dispersed on the surface of Mueller-Hinton agar (Oxoid, UK) and incubated at 37°C for 24 h. The isolates were assessed for sensitivity or resistance to particular antimicrobial agents such as penicillin G (10 μg), ampicillin (10 μg), amoxicillin (10 μg), cefuroxime (30 μg), ceftriaxone (30 μg), amikacin (30 μg), ceftazidime (30 μg), imipenem (10 μg), gentamicin (10 μg), ciprofloxacin (5 μg), and tetracycline (30 μg) based on inhibitory zones.

3.4. Polymerase Chain Reaction

The DNA was extracted from culture using a DNA purification kit (Hiper® Bacterial Genomic DNA Extraction Teaching Kit (India). After extraction, the DNA templates were stored at -20°C for further use. The primer sequences including F: (CCG GAA CAG AAG TTG TCG CTG GA) and R: (GGG CTC TCC TAC CGA CTT GAT C) were designed to allow for the amplification of the 533-bp fragment of the ureC1 gene. For PCR amplification, a 25-μL volume reaction mixture was used containing 12 μL of master mix (2x AmpMaster™ Taq), 9 μL of grade water, 1 μL of each primer (forward, reverse), and 2 μL of template DNA. PCR cycling for reaction mixture included initial melting at 94°C for 3 min, denaturing at 94°C for 1 min, annealing at 63°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 7 min. The final PCR product was run on the 1.5% agarose gel and observed under UV light.

4. Results

Overall, 480 wound samples were collected at Bolan Medical Complex Hospital, Quetta. There were 64 (13.3%) samples positive and 416 (86.6%) samples negative for *P. mirabilis*, as shown in Figure 1. Gender wise distribution showed that the wounds of male patients (n = 40; 8.3%) were more affected by *P. mirabilis* than the female patients' wounds (n = 24; 5%), as shown in Figure 2. Wound infections caused by *P. mirabilis* affected all age groups including children, teenagers, and older people. According to our study, the rate of infection with *P. mirabilis* was the highest in 16 - 30-year-old patients (n = 32; 6.7%), followed by 5 - 15-year-old patients (n = 24; 5%) and 30 - 50-year-old patients (n = 8; 1.60%), as shown in Figure 3. The results also showed that diabetic (n = 24; 5%) and surgical (n = 24; 5%) wounds were more affected by *P. mirabilis* than burn wounds (n = 16; 3.30%), as shown in Figure 4. *Proteus mirabilis* was identified through differential and selective media, Gram-staining, and biochemical tests, as shown in Table 1.

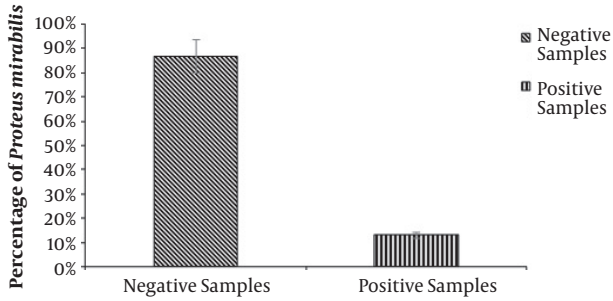

Figure 1. Positive and negative samples of *Proteus mirabilis* isolated from wounds

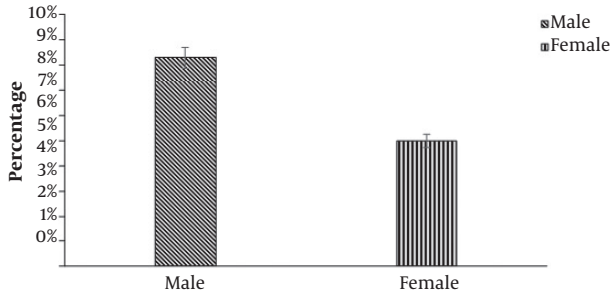

Figure 2. Gender wise distribution of *Proteus mirabilis* in patient's wounds

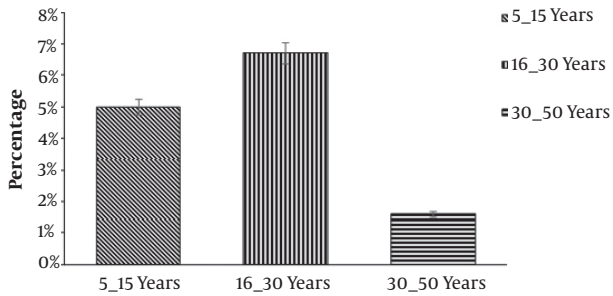

Figure 3. Age-wise distribution of *Proteus mirabilis* in patient wounds

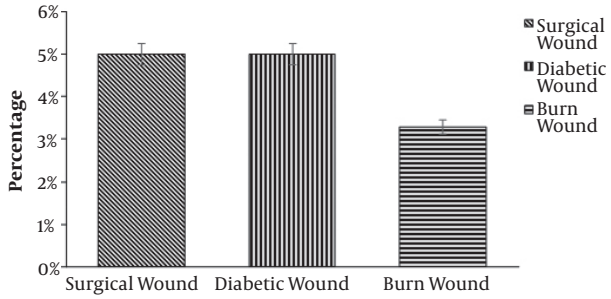

Figure 4. Distribution of *Proteus mirabilis* in different wound samples

4.1. Antimicrobial Susceptibility Test

Proteus mirabilis isolates were sensitive to gentamicin (n = 50; 78%) and amikacin (n = 53; 82.8%) while resistant to penicillin G (n = 58; 90%), ampicillin (n = 56; 87.5%), amoxicillin (n = 60; 93.7%), cefuroxime (n = 61; 95.3%), ceftriaxone (n = 57; 89%), ceftazidime (n = 59; 92.1%), imipenem (n = 62; 96.8%), ciprofloxacin (n = 55; 85.9%), and tetracycline (n = 59; 92%), as shown in Table 2.

Table 1. Biochemical Characterization of *Proteus mirabilis* Isolated from Wound Samples[a]

Proteus mirabilis	Values
Culture media	
MacConkey agar	Non-lactose fermenting, large, circular smooth colonies
Proteeae isolation medium (PIM) agar	Dark brown colonies with a zone of clearing in the medium
Gram staining	
Gram staining	-
Shape	Rods
Size	1 - 3 μm in length
	0.4 - 0.8 μm in width
Biochemical tests	
Motility	+
Indole	-
Citrate utilization	+/-
MR	+
VP	-
Urease	+
Catalase	+
Oxidase	-
Sugar fermentation tests	
Glucose	+
Sucrose	-
Sorbitol	-
Trehalose	+
Lactose	-
Mannitol	-
Dulcitol	-
Xylose	+
Inositol	-

[a] Positive (+), Negative (-), Variable (+/-).

4.2. Confirmation of P. mirabilis Through PCR

In the present study, the PCR-based identification of a specific gene was used to confirm *P. mirabilis*. All the isolates of *P. mirabilis* were used to produce a specific size of the 533-bp fragment of the ureC1 gene, as shown in Figure 5.

5. Discussion

Proteus mirabilis can cause both community and hospital-acquired infections, especially in immunocompromised patients. Based on the study by Brown (11),

Table 2. Antibiotic Susceptibility Pattern of *Proteus mirabilis* Isolated from Wound Samples

Classes	Antibiotics	Abbreviation	Resistant, No. (%)	Sensitive, No. (%)
Penicillin				
	Penicillin G	PEN	58 (90)	6 (9.3)
	Ampicillin	AP	56 (87.5)	8 (12.5)
	Amoxicillin	AMP	60 (93.7)	4 (6.2)
Cephalosporin				
	Cefuroxime	CXM	61 (95.3)	3 (4.6)
	Ceftriaxone	CTX	57 (89)	7 (10.9)
	Ceftazidime	CAZ	59 (92.1)	5 (7.8)
	Imipenem	IMP	62 (96.8)	2 (3.1)
Aminoglycoside				
	Gentamicin	GEM	14 (21.8)	50 (78)
	Amikacin	AMK	11 (17)	53(82.8)
Quinolones				
	Ciprofloxacin	CIP	55 (85.9)	9 (14)
Tetracycline				
	Tetracycline	TET	59 (92)	5 (7.8)

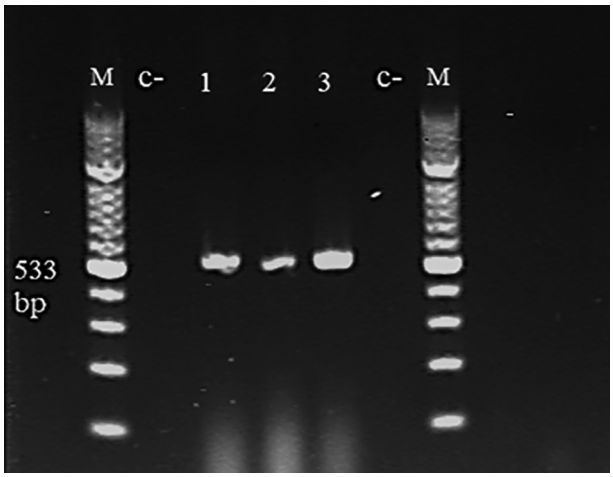

Figure 5. PCR-based identification of *P. mirabilis* from patient wounds

Proteus is the third main cause of hospital-acquired infections and a major contributor to the pathogenesis of wound infection. In the present study, 480 different wound samples were examined out of which, 13.3% were positive for *P. mirabilis* while 86.6% produced negative results. Among the positive samples, male patients' wounds (8.3%) were more infected with *P. mirabilis* then female patients' wounds (5%). Bahashwan and El Shafey (20) indicated in their study that males were more affected by *P. mirabilis* than females. This study showed that *P. mirabilis* affected all age groups, mostly 16 - 30 years-old (6.70%), followed by 5 - 10 years-old (5%) and 30 - 50 years-old (1.60%).

In this study, *P. mirabilis* was mostly found in surgical (5%) and diabetic (5%) wounds, followed by burn wounds (3%). Mohammed et al. (21) similarly reported in Nigeria that *P. mirabilis* was more frequently isolated from surgical wounds than burn and diabetic wounds. We identified *P. mirabilis* through differential media, Gram staining, and biochemical tests such as IMVIC, catalase, oxidase, sugar fermentation, citrate utilization, and urease and found results similar to the findings obtained by Cowan (22).

The control of wound infection has become more challenging due to the antibiotic resistance of infections. This study showed that *P. mirabilis* was sensitive to gentamicin and amikacin while resistant to penicillin G, ampicillin, amoxicillin, cefuroxime, ceftriaxone, ceftazidime, imipenem, ciprofloxacin, tetracycline. Mordi and Momoh (23) also found that all positive samples were resistant to tetracycline and erythromycin but sensitive to ciprofloxacin, ofloxacin, and gentamicin. In the present study, the PCR-based identification of a specific gene was used to detect *P. mirabilis*. All isolates of *P. mirabilis* were used to produce the specific size of the 533-bp fragment of the ureC1 gene; similar results were found by Abbas et al. (24).

Wound infection caused by *P. mirabilis* is a serious problem nowadays because bacteria have acquired resistance

to many antibiotics. We should take those measures for the treatment of wound infections caused by *P. mirabilis* that can give us better results in the future. We also need to work on different antibiotic-resistance genes and study the whole genomic sequences of *P. mirabilis* using PCR.

5.1. Conclusions

The pathogenesis of *P. mirabilis* in wound infection and its antimicrobial sensitivity are major problems worldwide. The use of aminoglycosides such as gentamicin and amikacin is effective against *P. mirabilis* that helps prevent the spread of infection and reduce the cost of treatment. PCR is one of the sensitive, timesaving, specific, and cost-effective ways for the identification of the pathogenic genes of *P. mirabilis*.

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Footnotes

Authors' Contribution: Umbreen Zafar performed the experimental work; Muhammad Kamran Taj and Imran Nawaz proposed and designed the research work; Imran Taj provided samples and analyzed data; Umbreen Zafar prepared manuscript; Muhammad Kamran Taj, Asma Zafar and Umbreen Zafar revised and approved the final manuscript.

Conflict of Interests: The author has no conflicts of interest.

Ethical Approval: This study was approved by the Ethics Committee of the Department of Microbiology, University of Balochistan, Quetta, and Bolan Medical Complex Hospital, Quetta, Balochistan (reference number UOB:2015-239).

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