Prevalence and Emergence of Multi Drug Resistance Among *Proteus* Species Isolated from Various Clinical Samples

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Abstract:
The worldwide problem of multidrug resistance is such that we are at the end of the pipeline of antibiotics. The aim of this study was to analyze prevalence of multidrug resistant *Proteus sp.* in clinical samples. *Proteus* is opportunistic pathogens commonly responsible for nosocomial infections often urinary tract infections, wounds infections and septic infections. In present study total 100 clinical samples from pus, urine, stool and blood were screened for isolation of *Proteus sp.* Total 21 suspected isolates were identified as belonging to *Proteus sp.* on the basis of morphological, cultural & biochemical characteristics. Antibiogram study of these isolates revealed that all these isolates are resistant to several antibiotics out of 16 antibiotics tested. Antibiogram of these isolates showed that Imipenem was found to be the most effective drug for *Proteus sp.*

Keywords:
Multidrug resistance, *Proteus sp.*, nosocomial infections.

Introduction:
Selecting the correct antibiotics for right treatment of bacterial infection is becoming increasingly complicated because most of the gram negative bacterial pathogens carry multiple resistances genes that make them responsible for global drug resistant problems (Edwin C. P. 2011). The worldwide excessive use of antibiotics in the treatment of Infectious bacterial diseases has led to the emergence and spread of Multi drug resistant problems (Sibanda T. et al., 2008). The spread of antimicrobial resistance among members of the *Enterobacteriaceae* is a significant clinical threat. *Proteus* spp. are part of *Enterobacteriaceae* family, turned researcher’s attention because of high occurrence in nosocomial infections and expanding profile of antibiotic resistance (Orhue O.P., 2014, Sibanda T. et al., 2008). *Proteus* sp. are opportunistic pathogens commonly responsible for urinary tract and wounds infections, bacteraemia and sepsis (Reslinski A. et al., 2005). It is widespread in the environment and makes up part of the normal flora of the human gastrointestinal tract. *Proteus* species are distinguishable from other genera on the basis of their ability to swarm across an agar surface.

Three species: *P. vulgaris*, *P. mirabilis*, and *P. penneri* are opportunistic human pathogens *Proteus* species are the major cause of diseases acquired
outside the hospital, where many of these diseases eventually require hospitalization. *P. mirabilis* causes 90% of Proteus infections. Proteus species, particularly *P. mirabilis*, is believed to be the most common cause of infection-related kidney stone, one of the most serious complications of unresolved bacteriuria. *P. mirabilis* has been implicated in meningitis, empyema, osteomyelitis and gastroenteritis. Also, it frequently causes nosocomial infections of the urinary tract (46%), surgical wounds (24%) and lower respiratory tract (30%). Less frequently, *Proteus* species cause bacteraemia (17%), most often in elderly patients. The phenomenal evolution and increase of multidrug-resistance of many bacterial pathogens is increasing and representing a growing public health problem in the world (Saleh A. B. *et al.*, 2013). *Proteus mirabilis* strains account for about 10% of uncomplicated urinary tract infections and they are about the fifth most common cause of nosocomial urinary tract infections and sepsis in patients.

**Material and methods:**

**Antibiotics:** Piperacillin / Tazobactam (PIT), Ceftazidime (CAZ), Ceftriaxone (CTR), Cefoperazone (CPZ), Cefuroxime (CFM), Cefepime (CPM), Amikacin (AK), Gentamicin (GEN), Norfloxacin (Nx), Nalidixic acid (NA), Ofloxacin (OF), Ciprofloxacin (CIP), Co-Trimoxazole (COT) (non lactam), Imipenem (IPM), Meropenem (MRP), Nitrofurantoin (NIT), Amoxyclov (AMC).

**Media:** Nutrient agar, Nutrient broth, MacConkey agar, Hi sensitivity agar

**Clinical samples:** Clinical samples of urine, pus, stool and blood samples were obtained from different pathology laboratories of Nagpur.

**Isolation of Proteus sp. from various clinical samples:**

A sample was immediately transferred to sterile nutrient broth for enrichment under aseptic condition and incubated at 37°C for 48 hrs. After 48 hours, loop full of culture from nutrient broth was streaked on selective media, Mac Conkey agar medium. Suspected colonies of *Proteus* sp. showing swarming growth and pink colour colony on Mackonkey medium were picked up and maintained on nutrient agar slant for further identification.

**Identification of isolates:**

Isolates were identified on the basis of morphological, cultural & biochemical characteristics and the results were compared with Bergey’s Manual of Determinative Bacteriology 9th edition as well as confirmed by biochemical identification using Vitek 2 System.

**Preparation of inoculums:**

A loopful of culture from slants was inoculated in 5 ml sterile nutrient broth and incubated at 37°C for 24hrs. Again loopful of culture from same broth was transferred to fresh 5ml of sterile nutrient broth and incubated at 37°C for 6-8 hrs. Turbidity was adjusted according to 0.5 McFarland
standards which were then used as inoculums which correspond to size of 1.5×10^8 CFU/ml.

**Antibiotic Susceptibility Test:**

About 15ml of sterile molten Hi sensitivity test agar medium poured in Petri dish at 50°C and mixed well. The medium was then allowed to solidify at room temperature. 0.1ml (0.5 McFarland standards) of inoculums was transferred on to the surface of agar medium with the help of micropipette under aseptic condition. It was spread uniformly all over the plate by using sterile L-spreader. Selected antibiotic discs with the help of sterile forceps were placed on Hi-sensitivity agar surface and pressed gently. Four equidistant antibiotic discs were placed per plate. Plates were kept undisturbed in a refrigerator for 1 hour for diffusion of antibiotics into the media and then shifted to incubator maintained at 37°C for 18-24hrs. After incubation all plates were examined for zone of inhibition. Isolates were considered susceptible, intermediate, or resistant to a particular antimicrobial agent on the basis of the diameters of the inhibitory zones that matched the criteria of the manufacturer’s interpretive table, which followed the recommendations of the performance standard for antimicrobial disk susceptibility test, CLSI (Bauer A.W. *et al.* 1966, CLSI 2007).

**Result and discussion:**

Total 100 Clinical samples of urine, pus, stool and Blood were collected from different pathology laboratories of Nagpur for isolation of *Proteus sp.* Total 21 suspected isolates of *Proteus sp.* were identified on the basis of morphological, cultural & biochemical characteristics and all of them were found to be *Proteus sp.* 12 isolates were associated with urine samples, 4 with pus and 5 was associated with stool. Percentage prevalence of *Proteus* sp. clinical samples is graphically illustrated in Figure.1. More than 57% *Proteus* sp. were found to be associated with urine samples, 23.80% and 19% with stool samples and pus samples respectively and none was isolated from blood samples.

Species wise distribution of Proteus is given in Table 1. *P. mirabilis* is most prevalent than *P. vulgaris* and most predominantly associated with urinary tract infections. Percentage for distribution of the various *Proteus sp.*, isolated from clinical samples are graphically illustrated in Figure.2. Percentage of isolates exhibiting resistance to antibiotic is given in Table 2.

From the above results it was revealed that amongst antibiotics tested, *Proteus sp.* showed highest resistance towards Nitrofurantoin i.e. 100% which was followed by 61.90% of Ceprofloxacin, 57.14% of Cefoperazone, 47.61% of Ceftazidime, 42.85% of Piperaillin, Amikacin and Ceftriaxone, 38.09% of Ofloxacin and Co-Trimoxazole, 33.33% of Gentamicin, Nalidixic Acid, 28.57%
of Amoxyclyave and 19.04% of Meropenem, Cefuroxime and Norfloxacin. But more interestingly Imipenem was found to be the most effective drug for Proteus sp. As none of the isolate exhibited resistance to it.

Three Proteus species (P. mirabilis, P. vulgaris and P. penneri) were identified to be responsible for causing infections in various anatomical sites. P. mirabilis was the most common species isolated, accounting for 75% of all the infections and hence responsible for the majority of Proteus infection. Proteus is therefore a common cause of wound infections in Navi Mumbai and other parts of Maharashtra, India (Saleh A.B. 2013). Our result agrees with these studies. Orhue O. Philips (2014) was demonstrated that there is high prevalence of Proteus mirabilis and Proteus morganii in UTI in the community under study and these strains of Proteus spp. are associated with higher antimicrobial resistance. Hence, multidrug-resistant Proteus mirabilis and Proteus morganii is becoming a growing public health problem (Orhue O.P. et al., 2014).

Jitendrakumar Pandey et al., (2013) revealed that P. mirabilis, P. vulgaris and P. penneri are the species concerned in Proteus infections; wounds recorded the highest incidence of Proteus infections at MGM Hospital, Navi Mumbai. They were found resistant to ampicillin, Netilline and Cefuroxime and Pefloxacin (Jitendra K. P. et al., 2013). Reslinski A. et al, (2005) was found that all of multidrug-resistant strains of Proteus were resistant to penicillins and cephalosporins, 98.9% to co-trimoxazole, 77.7% to quinolones, 63.8% to tetracyclines, 38.5% to aminoglycosides, 19.3% to monobactams and 3.4% to carbapenems. Almost 25% multidrug-resistant Proteus sp. produced ESBL (Reslinski A. et al., 2005).

Finding of Gebre et al., (2007) revealed that ciprofloxacin was highly effective antimicrobial agent in vitro against majority of the bacterial isolates (Gebre et al., 2007). The findings of our study showed that Imipenem was found to be the most effective drug for Proteus sp. Apurva K. Pathak (2011) found fair correlation with our studies but with other members of Enterobacteriaceae in which Enterobacter spp. showed resistance towards the six classes of antibiotics and Klebsiella spp. And Salmonella spp. towards five classes of antibiotics (Apurva K. P. 2011).

M. M. Salem-Bekhit et al., (2012) study showed that there is an emergence of Vancomycin-resistant enterococci along with increased rate of multidrug-resistant enterococci (MM S.B. et al., 2012 ). In another study by Mercedes et al, (2013) reported that increasing frequency and the rapid spread of multidrug resistance among the Enterobacteriaceae (Mercedes et al, 2013). R. Saito et al., (2007) concluded that the majority of ciprofloxacin-resistant P. mirabilis isolates exhibited multi resistant due to previous use of fluoroquinolones and production of extended-spectrum b-lactamases (R. Saito
et al, 2007). R. M. Mordi et al., (2009) were suggested that the use of the floroquinolones and gentamycin as the antibiotic of choice in wound infections as they continue to be effective against Proteus infections (R. M. Mordi et al., 2009).

**Table 1:** Distribution of the various Proteus species isolated

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total (100)</th>
<th>Urine(50)</th>
<th>Stool(22)</th>
<th>Pus(20)</th>
<th>Blood(08)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vulgaris</em></td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>16</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2:** Antibiotic resistance pattern among isolates of Proteus spp.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Antibiotics</th>
<th>Concentration</th>
<th>% of isolates showing resistance(n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Imipenem</td>
<td>10 mcg</td>
<td>9.52(2)</td>
</tr>
<tr>
<td>2.</td>
<td>Meropenem</td>
<td>10 mcg</td>
<td>19.04(4)</td>
</tr>
<tr>
<td>3.</td>
<td>Piperacillin/Tazobactam</td>
<td>30 mcg</td>
<td>42.85(9)</td>
</tr>
<tr>
<td>4.</td>
<td>Cefoperazone</td>
<td>30 mcg</td>
<td>57.14(12)</td>
</tr>
<tr>
<td>5.</td>
<td>Amikacin</td>
<td>30 mcg</td>
<td>42.85(9)</td>
</tr>
<tr>
<td>6.</td>
<td>Nitrofurantoin</td>
<td>300 mcg</td>
<td>100(21)</td>
</tr>
<tr>
<td>7.</td>
<td>Gentamicin</td>
<td>10 mcg</td>
<td>33.33(6)</td>
</tr>
<tr>
<td>8.</td>
<td>Ceftazidime</td>
<td>30 mcg</td>
<td>47.61(10)</td>
</tr>
<tr>
<td>9.</td>
<td>Cephalaxacine</td>
<td>30 mcg</td>
<td>61.90(13)</td>
</tr>
<tr>
<td>10.</td>
<td>Ofloxacin</td>
<td>30 mcg</td>
<td>38.09(8)</td>
</tr>
<tr>
<td>11.</td>
<td>Co-Trimoxazole</td>
<td>30 mcg</td>
<td>38.09(8)</td>
</tr>
<tr>
<td>12.</td>
<td>Ceftriaxone</td>
<td>30 mcg</td>
<td>42.85(9)</td>
</tr>
<tr>
<td>13.</td>
<td>Norfloxacin</td>
<td>30 mcg</td>
<td>33.33(7)</td>
</tr>
<tr>
<td>14.</td>
<td>Nitrofurantoin</td>
<td>30 mcg</td>
<td>28.57(6)</td>
</tr>
<tr>
<td>15.</td>
<td>Cefuroxime</td>
<td>30 mcg</td>
<td>19.04(4)</td>
</tr>
<tr>
<td>16.</td>
<td>Norfloxacin</td>
<td>10 mcg</td>
<td>19.04(4)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicates number of isolates.

**Figure 1:** Graph showing % prevalence of *Proteus sp.* in clinical samples.

**Figure 2:** Graph showing % Distribution of the various *Proteus species* isolated
Conclusion:

*Proteus* is opportunistic pathogens commonly responsible for nosocomial infections often urinary tract infections, wounds infections and septic infections. The development of drug resistance is therefore major concern in the management of infections associated with *Proteus* sp. The most prevalent *Proteus* species is *P. mirabilis* which has exhibited resistance to many antibiotics tested. Interestingly Imipenem was found to be effective against all the isolates but *Proteus* species exhibited resistance against other antibiotics. It is therefore empirical to understand the antibiogram as routine practice for management of *Proteus*.

References:


Mercedes Delgado-Valverde, Jesus Sojo-Dorado, Alvaro Pascual, Jesus Rodriguez Bano (2013) Clinical management of infections caused by multidrug-reseistant Enterobacteriaceae, Therapeutic Advances in Infectious Disease


