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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 World wide 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 bacter uria. *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), Amoxyclav (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 clinicalSamples:

A sample was immediately transferred to sterile nutrient broth for enrichment under aseptic condition and incubated at 37^{0}C for 48 hrs. After 48 hours, loop ful 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 Bacteriology9th 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^{0}C for 24hrs. Again loopful of culture from same broth was transferred to fresh 5ml of sterile nutrient broth and incubated at 37^{0}C for 6-8 hrs. Turbidity was adjusted according to 0.5 McFarland

standards which were then used as inoculums which correspond to size of1.5×108 CFU/ml.

Antibiotic Susceptibility Test:

About 15ml of sterile molten Hi sensitivity test agar medium poured in Petri dish at 50^{0}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^{0}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 21suspected 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*in clinical samples is graphically illustrated in Figure.1. More than57% *Proteus sp.* were found to be associated with urine samples, 23.80% and19% 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 Amoxyclave 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

Samples	Total (100)	Urine(50)	Stool(22)	Pus(20)	Blood(08)
P. vulgaris	5	2	2	1	0
P.mirabillis	16	10	3	3	0

Table 2: Antibioticresistance pattern among isolates of Proteus *spp.*

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

Figures in parenthesis indicates number of isolates

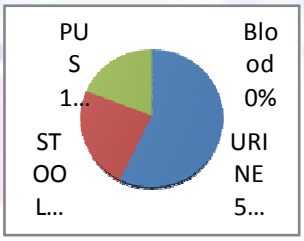

Figure1: Graph showing % prevalence of *Proteus sp.* in clinical samples.

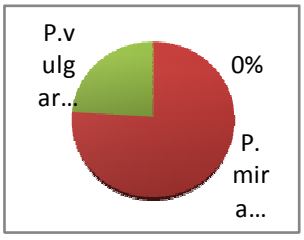

Figure 2: Graph showing % Distribution of the various *Proteus species* isolated

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.*

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