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Original Research Paper

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MBL PRODUCING P.PENNERI - A MAJOR THREAT TO THE HOSPITAL

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ABSTRACT

Introduction: *Proteus spp*. has been the significant cause of wound infections as they commonly colonize the wound. Simultaneously multiple drug resistance mechanisms also pose a therapeutic challenge. *P.penneri* is commonly misidentified as *P.mirabilis* which is multidrug resistant. **Aim and objectives:** The current study was conducted to determine the prevalence of MBLs in *P.penneri* among wound infections at a tertiary care hospital from Western Maharashtra. **Material and Methods:** Specimens collected from wounds of OPD and IPD patients were examined by standard bacteriological methods. All *Proteus* isolates were subjected to Antimicrobial susceptibility and MBL production test as per CLSI guidelines. **Results:** Total 1826 wound samples were screened over the period of May 2017 to July 2018. Overall prevalence of *Proteus spp*. was 7.12%, *P.mirabilis* being the commonest. Among all isolates 53.68% and 37.04% of *P.mirabilis* and *P.vulgaris* were ESBL producers, respectively. The rate for MBL production was 11.58% and 0% for *P.mirabilis* and *P.vulgaris*, respectively. On the contrary 75% of *P.penneri* isolates were ESBL producers and 12.5% were MBL producers. **Discussion and conclusion:** Emergence of ESBL and MBL producers is of special concern as *Proteus spp*. is intrinsically resistant to tigecycline and colistin. Identification of *P.penneri* from clinical specimens is necessary, due to its multidrug resistance which makes clinical treatment extremely difficult. This will limit its control and eradication especially from wound infections as they are common colonizers. Therefore isolation of such beta lactamases producing *P.penneri* shall be considered as an alarming sign to control the spread of this superbug.

KEYWORDS : Proteus, Wound infections,ESBL, MBL

INTRODUCTION:

The genus *Proteus* has been frequently recovered from humans in different infections. It has been significant cause of both urinary tract infections and wound infections including nososcomial infections. Currently genus *Proteus* has different species causing human infections namely *P.mirabilis*, *P.vulgaris*, *P.penneri*, *P.hauseri*; *P.mirabilis* being the most predominant species recovered. *P.vulgaris* is more commonly recovered from infections, especially in immunocompromised patients. There has been documented infections by *P.penneri* also, most frequent being urinary tract infection and wound infections. Few isolated case reports are found in literature on bacteremia. [1]

P.penneri belongs to Proteus biogroup 1, which are indole negative, salicin negative and often fails to produce H_2S in triple sugar iron test. *P. penneri* is rarely encountered in clinical laboratories, often misidentified as *P.mirabilis*. It can be easily differentiated from other *Proteus spp*. by simple test like chloramphenicol susceptibility test. *P. penneri* is chloramphenicol-resistant, whereas other indole-negative *Proteus spp*. are chloramphenicol susceptible. Whenever indole negative *Proeus spp*. is isolated in laboratory chloramphenicol susceptibility test should be performed. [1,2]

P. penneri is capable of causing significant nosocomial infections. *P.penneri* has tendency towards multidrug resistance; naturally resistant to penicillin, amoxicillin, cephalosporins due to the ability of producing inducible ß-lactamases. [3,4]

Extended spectrum beta lactamase (ESBL) producing Proteus strains had been isolated from different sites of infections, which became a cause of concern in various hospitals as management viewpoint. [5,6] MBL production, further adds to the problem. The only remedy for these strains is Polymyxins and *Proeus spp*. are intrinsically resistant to Polymyxins which makes clinical treatment extremely difficult. We aimed this study to determine the MBL production in *P.penneri* and MDR nature among wound infections.

MATERIAL AND METHODS:

We conducted a study during May 2017 to July 2018 which was prospective laboratory based. All wound specimens from both- inpatients as well as outpatients were included in the study. Specimens received at laboratory were inoculated on routine media i.e. Blood agar, MacConkey agar and incubated overnight at 37^{0}C. Clinical isolates were identified to the species level by standard bacteriological methods. [1] Especially Chloramphenicol susceptibility test was used to differentiate *P.penneri* from other *Proteus spp*. Antimicrobial susceptibility tests were done by Kirby-Bauer disc diffusion test as per CLSI guidelines. [7]

ESBL production test:

Screening of ESBL producing strains:

Isolates resistant to ceftazidime(<22mm) and/or ceftriaxone (<25mm) and/or cefotaxime (<27mm) were screened for ESBL production

Confirmatory test for ESBL production:

This was done by Combined disc diffusion method. Ceftazidime(30μg) and cefotaxime(30μg) discs alone were used along with combination with clavulanic acid. Isolates showing increase in zone diameter of >5 mm with clavualnate combined disc compared to that of disc alone were considered as ESBL producers . [7]

Carbapenase production test:
Modified Hodge test: 0.5 McFarland of the *E.coli* ATCC 25922 was diluted to 1:10 dilution. This suspension was streaked on Mueller Hinton(MH) agar. Thereafter Meropenem (10 µg) disc was placed in the center of the plate. The test isolate was streaked in straight line from disc to the edge of the plate. Interpretation of test was done according to CLSI guidelines.

MBL detection test: Combined disc diffusion test [8]

Test organisms were inoculated on to plates with Muller Hinton agar as recommended by the CLSI guidelines. One 10 µg Imipenem disc and one Imipenem EDTA disc was placed. The inhibition zones of the Imipenem and Imipenem EDTA discs were compared at 16 to 18 hours of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was >7mm than the Imipenem disc alone, it was considered MBL positive.

RESULTS:

Out of total 1826 wound samples total culture positivity was 1457(79.79%). Most of the specimens grew mixed organisms. Bacteriological profile of wound infections is as depicted in table 1. *S.aureus* was the predominant pathogen isolated followed by *P.aeruginosa* and *A.baumanii*. *Proteus spp*. contributed to 7.12% of total profile. Among all *Proteus spp*. subjected to detailed biochemical analysis, eight (6.15%) isolates of *P. penneri* were identified (table2).

All Proteus isolates showed different antibiogram. *P. penneri* isolates exhibited multidrug resistance to most of the antibiotics followed by *P. mirabilis*.(Table 3). ESBL production rate was much higher in *P.penneri* as compared to other *Proteus spp*. 75% of *P.penneri* were ESBL producers while rate was low in *P.mirabilis*(53.68%) and *P.vulgaris*(37.04%). MBL production rate was 11.58% for *P.mirabilis* while 12.50% for *P.penneri* .while MBL production rate in *P.vulgaris* was 0.

DISCUSSION:

Proteus spp. are one of the common cause for wound infections. Especially *P.penneri* usually missed during routine analysis. There are very limited studies conducted on *P.penneri*. Thatswhy our study was focused on evaluation of resistance pattern among *Proteus spp*. with special reference to *P.penneri*. When we analyzed detailed biochemical characteristics among *Proteus spp*. , 6.15% *P. penneri* isolates were identified. Similar prevalence has been reported by Prasad et al [9](6.25%), while higher prevalence was reported by Pal et al [10](7.92%) and Kishore et al [2](13.1%).

P. penneri isolates exhibited multidrug resistance to most of the antibiotics followed by *P. mirabilis*.(Table 3) Feglo et al [11], Kishore et al [2] and Senthamarai *et al* [12] also reported high MDR status in *P. penneri* isolates. Resistance was almost 100% for cephalosporins , may be due to their ability to produce different inducible beta lactamases. Our study observed higher ESBL production rate in *P.penneri (75%)* as compared to other *Proteus spp*. These findings were in discordance with Pandey et al [13] in which ESBL production rate was 8.33%. this discordance infindings may be due to limited studies conducted focusing on *Proteus spp*. This suggests, need of further studies highlighting resistance pattern among *Proteus spp*.

Carbapenems remains the drug of choice in ESBL producing strains. In the Current study observed resistance pattern was more towards carbapenems. Imipenem (25%) has less susceptibility as compared to meropenem(87.5%). Though Meropenem showing good sensitivity , susceptibility has been less in *P.penneri* as compared to other Proteus spp. Overall Meropenem resistance was 12.5%, similar finding has been found in different studies such as Pal et al [10], Pal-Hooja et al.[14] On the contrary, very low resistance has been reported by Senthamarai et al.[12]

On further evaluation for MBL production we found none of *P.vulgaris* as MBL producers, while scenario was quite serious in *P.penneri* and *P.mirabilis*. MBL production rate was 11.58% for *P.mirabilis* while 12.50% for *P.penneri* . Reviewed literature did not report MBL production exclusively by *P.penneri*. Certain studies such as Oberoi et al [15] and Devaraju et al [16] observed none of Proteus spp. were MBL producers. Only remedy for MBL production is Polymyxins and *Proteus spp*. are intrinsically resistant to Polymyxins which leads to clinical management difficult. This trend of increase in MBL production rate is an alarming sign for recent future.

The present study has certain limitations: as due to resource unavailability, we were not able to evaluate genotypic pattern of MBL producing strains.

CONCLUSION:

P.penneri is capable of causing significant nosocomial infections due to their mutidrug resistance. Identification of such beta-lactamase producing *P.penneri* can be done by simple test like Chloramphenicol susceptibility. MBL production among such MDR *P.penneri* strains becomes a therapeutic challenge for clinicians. Thus efforts should be made to identify *P. penneri* infections. There is a need for active surveillance to detect MBL producing *P.penneri* which can be considered as an alarming sign to control the spread of this superbug. Strengthening infection control practices is also need of hour so as to prevent emergence of such nosocomial threats in the hospital settings.

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Table 1: Bacteriological profile

Sr.No.	*Organism*	% of isolation
1	*Staphylococcus aureus*	316(17.31%)
2	*Pseudomans aeruginosa*	314(17.20%)
3	*Acinetobacter baumanii*	284(15.55%)
4	*E.coli*	203(11.12%)
5	*Klebsiella pneumoniae*	171(9.36%)
6	*Proteus spp.*	130(7.12%)
7	*Coagulase Negative Staphylococci(CoNS)*	123(6.74%)
8	*Citrobacter spp.*	112(6.13%)
9	*Enterobacter spp.*	74(4.05%)
10	*Enterococcus spp.*	64(3.50%)
11	*Streptococcus spp.*	16(0.88%)
12	*Other spp.*	16(0.88%)

Table 2: Proteus Isolates Differentiation:

Sr.No.	*Proteus spp*(n=130)	No.of Isolates(%)
1	*P.mirabilis*	95(73.07)
2	*P.vulgaris*	27(20.77)
3	*P.penneri*	8(6.15)

Table 3: Susceptibility Profile of Proteus spp.

Antibiotic Groups	Antibiotics	P.mirabilis (n=95)	P.vulgaris (n=27)	P.penneri (n=8)
Penicillins	Ampicillin	17(17.89%)	9(33.33%)	1(12.5%)
	Ampicillin-sulbactam	37(38.95%)	14(51.85%)	4(50%)

	Amoxicillin-clavulanate	12(12.63%)	4(14.81%)	6(75%)
	Piperacillin	59(62.11%)	25(92.59%)	4(50%)
	Piperacillin-tazobactam	78(82.11%)	27(100%)	6(75%)
Cephalosporins	Ceftazidime	31(32.63%)	12(44.44%)	1(12.5%)
	Ceftriaxone	38(40%)	16(59.26%)	0
	Cefotaxime	35(36.84%)	15(55.56%)	0
	Cefuroxime	31(32.63%)	14(51.85%)	0
	Cefoperazone	32(33.68%)	16(59.26%)	0
	Cefoperazone-sulbactam	76(80%)	27(100%)	6(75%)
Carbapenems	Meropenem	82(86.32%)	26(96.30%)	7(87.5%)
	Imipenem	46(48.42%)	14(51.85%)	2(25%)
Fluoroquinolones	Ciprofloxacin	36(37.89%)	22(81.48%)	2(25%)
Folate inhibitors	Cotrimoxazole	29(30.53%)	7(25.93%)	3(37.5%)

Table 4: ESBL & MBL production rate among Proteus spp.:

	P.mirabilis (n=95)	*P.vulgaris* (n=27)	*P.penneri* (n=8)
ESBL	51(53.68%)	10(37.04%)	6(75%)
MBL	11(11.58%)	0	1(12.5%)

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