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A Study of Bio-Film Production and Antibiotic Sensitivity Pattern of Proteus Species Isolated From Pus and Urine Samples

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Abstract: Proteus species is a member of the Enterobacterieceae family. It is identified as one of the etiological agents of hospital acquired infections. The expanding profile of antibiotic resistance among Proteus species is a clinical threat. It is also one among the biofilm forming organisms. The present study was undertaken to determine biofilm production in Proteus species by three different phenotypic methods and to detect Extended spectrum betalactamase and Amp C betalactamase production among the isolates and to know the distribution of resistant strains in biofilm producing and non-producing isolates. A total of 100 proteus isolates from different samples were screened for biofilm production by Congo red agar (CRA) method, Tube method (TM) and Tissue culture plate (TCP) method. Antibiotic susceptibility was determined by Kirby Baeur's disc diffusion method following CLSI guidelines. ESBL and AmpC betalactamase production was detected by disc potentiation tests. Out of 100 Proteus isolates, 71 were P.mirabilis and 29 were P. vulgaris. Among them 69 were biofilm producers. ESBL production was detected in 62% and AmpC betalactamase in 44% of isolates and 28% were multidrug resistant strains. Resistance is more noticed in biofilm producers than non-producers. As there is increasing pathogenicity of Proteus species, they should be identified to species level and resistance pattern to be screened by simple, reliable and preferably inexpensive methods.
Keywords: Antibiogram, Biofilm, Proteus species.

INTRODUCTION

Proteus species are widely disseminated in the environment. They live in soil and water and play an important role in the natural environment decomposing organic material of the animals. These are also one among the commonly implicated pathogens in hospitals as well as a cause of community acquired infections. Owing to their varied habitats these pathogens have a diverse mode of transmission and hence can cause infection in different anatomical sites of the body [1-4].

Proteus species are the important etiological agents in Urinary tract infections in both community acquired and nosocomial infections. They may also contribute to infections of wound, respiratory tract, surgical site, diabetic ulcers, burns and also to other infections[1,5].

An important virulence factor of these bacteria is the ability to form biofilm. The biofilm structures preserve bacteria from unfavourable influence of the environment conditions and facilitates distribution of nutritional agents. Biofilm protects bacteria from immune response of the host, decreases antibiotic and antibody penetration. There is increasing evidence for the role of bacterial biofilm in various wound and urinary tract infections[6]. Biofilm formed on the abiotic surfaces is believed to be the major cause (65%) of nosocomial infections[1].

Selecting the correct antibiotic for the treatment of bacterial infection is becoming increasingly complicated because most of the gram negative bacterial pathogens carry multiple resistance genes that make them responsible for global drug resistance problem. The worldwide excessive use of antibiotics in the treatment of infectious bacterial diseases has led to the emergence and spread of multi drug resistant strains.

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 [7,8].

Multidrug resistant P. mirabilis has created serious concern in treatment of catheter associated UTI infections. The colonization of catheters and chronic wounds by biofilm producing strains of Proteus species caused treatment failure due to antibiotic resistance [8,9].

In the present study an attempt was made to isolate and identify the different Proteus species in pus and urine samples. The ability of biofilm production and the antibiotic resistance patterns were also studied.

MATERIALS AND METHODS

The present study was undertaken in the Department of Microbiology, Rangaraya Medical College, and Kakinada from Dec 2015 to Oct 2017. A total of 100 proteus isolates, 46 from pus and 54 from urine samples were studied for biofilm production and antibiotic resistance patterns.

Antibiotic susceptibility was done by Kirby-Bauer's disc diffusion method following CLSI guidelines. All the isolates which were resistant to cefotaxime were confirmed for ESBL production by Disc potentiation test using cefotaxime and cefotaxime + clavulanic acid. A difference of 5mm or more than 5mm increase in zone size with Clavulanic acid is taken as positive for ESBL production.

The isolates which were resistant to cefoxitin were confirmed for AmpC betalactamase production by Disc potentiation test using Ceftazidime and ceftazidime + boronic acid. Interpretation of disc potentiation test was based on the enlargement of the growth-inhibitory zone diameter (by greater than or equal to 5 mm) around a disc containing a ceftazidime + boronic acid.

All the Proteus isolates were screened for biofilm production by three phenotypic methods i.e. Congo red agar (CRA) method, tube method (TM) and Tissue culture plate (TCP) method.

RESULTS

Out of 100 proteus isolates, the most common species isolated was P. mirabilis (71) followed by P. vulgaris (29). Among them 87 were biofilm producers by CRA method, 79 by tube method and 69 by TCP method. As TCP method was the gold standard for biofilm production, the number positive by this method were taken for comparison in latter discussion. 70% of P. mirabilis and 65% of P. vulgaris are biofilm producers by this method.

Table I shows distribution of resistant strains among Proteus species. 54.92% and 40.84% 0f P.mirabilis and 79.31% and 51.72% of P.vulgaris were found to be ESBL and AmpC β-lactamase producers respectively. Isolates which were resistant to at least three different classes of antibiotics were taken as multi drug resistant. The isolates that were resistant to amikacin which were also ESBLs and AmpCs i.e Proteus mirabilis (21) and P. vulgaris (7) were treated as Multidrug resistant strains.

Table II shows distribution of ESBLs and AmpCs among Biofilm producing strains of Proteus isolates. More number of resistant strains was noticed among biofilm producers when compared to biofilm non-producers

Table-I: Distribution of Resistant strains in Proteus species

Species	ESBLs		AmpCs		MDR	
	No.	%	No.	%	No.	%
P. mirabilis (n=71)	39	54.92	29	40.84	21	29.57
P. vulgaris (n=29)	23	79.31	15	51.72	7	24.13
Total (100)	62	62	44	44	28	28

Table-II: Distribution of ESBLs and AmpCs among biofilm producing Proteus strains

	ESBL positive (n=62)	AmpC positive (n=44)
Biofilm producers (n=69)	55 (79.71%)	38(55.07%)
Biofilm non-producers (n=31)	7(22.58%)	6(19.35%)

DISCUSSION

Infections with Proteus have been reported with increasing frequency. The pathogenic role of Proteus is now well established and the clinical significance of Proteus species continues to increase. Because there is increasing pathogenicity of these organisms, Proteus should be identified to the species level by simple, reliable and preferably inexpensive methods.

They are intrinsically resistant to polymyxins, colistins and various other groups of antimicrobials along with evidence of ESBL and AmpC β lactamase production. Many of the proteus species are commonly resistant to antibiotics that are being indicated for other Enterobacteriaceae of clinical significance. In addition, biofilm function as a penetration barrier to antibiotics and hence the high level of resistance.

In the present study 100 strains of Proteus were isolated and the results obtained were compared with other studies and discussed as follows.

Out of 100 Proteus isolates screened for Biofilm production by TCP method 69(69%) were biofilm producers, which was nearly correlating with the study of Mohammad Zubair *et al.* [9] who reported 80% of biofilm producers. Joanne Kwiecinska-Pirog *et al*. [1] and Houshang Shikh *et al*. [10] had reported 100% and 93.47% respectively. The sample size in these studies was too small which might be the reason for high positivity.

Antibiotic susceptibility of Proteus species showed multidrug resistance. They showed maximum resistance to ampicillin (88%) followed by Cotrimoxazole (84%) and Cefotaxime (75%). All the strains were 100% sensitive to Carbapenems followed by Piperacillin/Tazobactum (81%), Amikacin (72%), Ciprofloxacin (69%), and Gentamicin (66%).This study correlates with the studies by Zineb Leulmi *et al*. [11] Algeria, Patrick Kwane Feglo *et al*. [12] and Orhue O Phillips *et al*. [13] who also showed maximum resistance to Ampicillin, Cefotaxime and Co-trimoxazole

Variability in the antibiotic susceptibility pattern of Proteus species has been observed by various other studies which positively reflect the different protocols and panels of antibiotics being used in different hospitals.

The present study showed 54.92% of P. mirabilis and 79.31% of the P. vulgaris were ESBL producers. The percentage of ESBLs in P. mirabilis correlates with that of Jitendra K Pandey *et al.* [14]. When compared among the two species the percentage of ESBL production in P. vulgaris was more than P. mirabilis in our study which correlates with that of Patrick k Feglo *et al*. [12].

AmpC betalactamase production was observed in 54.92% of P. mirabilis and 51.72% of P. vulgaris correlating with one study from Chennai by J Vinoth *et al*. [15] which shows 35% and 52% respectively. Similar to ESBL production, AmpC betalactamases were also more in P. vulgaris when compared to P. mirabilis. In other studies there was no data regarding AmpC betalactamase production in P. vulgaris.

CONCLUSIONS

The susceptibility pattern of Proteus strains isolated from our hospital revealed that majority of isolates was simultaneously resistant to atleast four antibiotics routinely used in our hospital. The high levels of betalactamase production and multidrug resistance of the isolates of Proteus may be an indication of resistance among the enterobacteriaceae. These species are potential causes of infections and reservoirs of resistance genes that could be transferred to other bacterial pathogens.

Biofilm production by Proteus strains found to play key role in multiple drug resistance phenomenons in this study. Hence proper detection of bacterial ability to form biofilm seems to be crucial aspect of medical investigation. Species identification and surveillance of antimicrobial resistance is essential in management and control of infections.

Since indiscriminate use of antibiotics leads to a higher prevalence of resistant bacteria which is very common in developing countries like India, strict implementation of appropriate infection control measures and the formulation of antibiotic policy must be done to prevent development and spread of resistant strains.

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