INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

A study on the antibiotic susceptibility pattern of *Proteus* spp among various samples.

Senthamarai S*, Sivasankari S., Anitha C, Kumudavathi MS, Amshavathani SK, Venugopal V and Thenmozhi Valli P R.

Department of Microbiology, Meenakshi Medical College and Research Institute, Enathur, Kanchipuram, Tamilnadu, India - 631 552.

Abstract

This study was done to evaluate the frequency of isolation of *Proteus* spp among various clinical samples and to know its susceptibility pattern in our tertiary care hospital, Kanchipuram. Total of 121 clinical isolates of *Proteus* spp were collected from wound, pus, sputum, urine, blood and body fluids. All the samples were collected with aseptic precautions and inoculated onto nutrient agar, blood agar, MacConkey agar and incubated at 37° c, overnight. The colonies were tested for biochemical tests and antibiogram. ESBL screening was done using 3rd generation cephalosporin's and confirmed combined double disc test. Among the 3972 total clinical samples 121 isolates of *Proteus* spp were isolated (3.04%). Urine (50.4%) and pus (44.6%) were the predominant sample of isolation. Male (63.6%) were commonly affected than female (36.4%). *Proteus mirabilis* was more commonly isolated than *Proteus vulgaris*. They were highly sensitive to imipenem, piperazillin-tazobactum and ofloxacin. *Proteus* spp were highly resistant to Ampicillin and amoxycillin-clauvulanic acid. The resistant pattern of *Proteus* spp to 3rd generation cephalosporins were –cefuroxime (37.1%), ceftriaxone (33.8%), Ceftazidime (26.4%) and cefotaxime(15.7%). 24.8% of *Proteus* spp were ESBL producers. Conclusion: This study highlights the prevalence of *Proteus* spp, its susceptibility pattern and ESBL production, in our tertiary care hospital, Kanchipuram.

Key words: *Proteus*, ESBL, susceptibility.

INTRODUCTION

Proteus species are gram negative bacilli belonging to family Enterobacteriaciae [1]. They are widely distributed in the environment and also as a part of normal flora of intestinal tract. This pathogen has various mode of transmission, and hence can cause infections in different anatomical site, and also they cause variety of community and hospital acquired infections [2]. It rank as 3rd cause of health care associated infections and the reported prevalence of *Proteus* infections were between 9.8% and 14.6 % [3]. As like other Enterobacteriaciae species, the antibiotic resistance due to the frequent and inappropriate use of antibiotics is a significant growing public health problem and the various resistance genes and MDR in *Proteus* spp needs for a regular review of its sensitivity pattern to decide about the treatment of these infections [2, 4]. *Proteus* species is known uropathogen especially causing urine tract infections in catheterized patients and those with urinary tract abnormalities[5]. It may lead to pyelonephritis, stones, fever and bacteraemia. [6]

Considering these above background, this study was done to evaluate the frequency of isolation of *Proteus* spp among various clinical samples and to know its susceptibility pattern in our tertiary care hospital, Kanchipuram.

MATERIALS AND METHODS

This study was conducted in the microbiology department, Meenakshi Medical College and Research Institute at Kanchipuram during the period of February 2013 to April 2014. Total of 121 clinical isolates of *Proteus* spp were collected from wound, pus, sputum, urine, blood and body fluids.

Demographic data were recorded prior to sample collection from patients. Ethical clearance was obtained from institutional ethical committee.

All the samples were collected with aseptic precautions and inoculated onto nutrient agar, blood agar, MacConkey agar and incubated at 37° c, overnight. The colonies were tested for the standard biochemical reactions for *Proteus* spp. [7]. The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion technique with commercially available disc (Hi media) on Muller Hinton agar. *E.coli* ATCC 25922 was used as control and results were interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines. Those strains showed resistance to 3rd generation cephalosporins were subjected to ESBL detection methods using combined disc diffusion test. (CLSI-2013)[8]

ESBL Screening (CLSI-2013) [8]

Screening of *Proteus* spp. for ESBL production was performed according to the procedures as recommended by the CLSI, using indicator cephalosporins, ceftriaxone (30µg), ceftazidime (30µg), and cefotaxime (30µg). Isolates exhibiting zone size ≤ 25 mm with ceftriaxone ≤ 22 mm for ceftazidime and ≤ 27mm with cefotaxime were considered as ESBL producer.

Phenotypic Confirmatory Test for ESBL: (Combined Disc Diffusion Method) (CLSI-2013) [8]

0.5 McFarland turbidity standard suspension was made from the colonies of *Proteus* spp. isolate. Using this inoculum, lawn culture was made on Muller Hinton Agar (MHA)plate. Discs of Ceftazidime and Ceftazidime + Clavulanic acid (30 mcg/10 mcg) were placed aseptically on the surface of MHA. The distance of 15 mm was kept between the disc and overnight incubation was done at 37°C. An increase of ≥ 5 mm in zone diameter of Ceftazidime + Clavulanic acid in comparison to the zone diameter of Ceftazidime alone confirmed the ESBL production by the organisms.

RESULTS

Among the 3972 total clinical samples 121 isolates of *Proteus* were isolated (3.04%). Urine (50.4%) and pus (44.6%) were the predominant sample of isolation.(Table-1). Male (63.6%) were commonly affected than female (36.4%).(Table-2). *Proteus mirabilis* was more commonly isolated than *Proteus vulgaris*.(Figure-1) They were highly sensitive to imipenem, piperazillin-tazobactum and ofloxacin. *Proteus* spp were highly resistant to Ampicillin and amoxicillin-clauvulanic acid. The resistant pattern of *Proteus* spp to 3rd generation cephalosporins were – Cefuroxime (37.1%), Ceftriaxone (33.8%) , Ceftazidime (26.4%) and Cefotaxime(15.7%). 24.8% of *Proteus* spp were ESBL producers.(Chart-1).

DISCUSSION

Despite the advent of various antimicrobials, nowadays, the community acquired and health care associated infections are relatively frequent with *Proteus* species. Its resistance to various groups of antibiotic has been increasing, which needs a continuous survey for better therapeutic response. There are limited studies and documented informations of infections caused by various species of *Proteus* related to patients demographies and its antibiotic resistant pattern in our area.

In this present study, the prevalence rate of *Proteus* among various clinical specimens is 3.04%. The same has been reported with Bahashwan *et al* (3%)[8]. Slightly lower prevalence rate 1.12%, has been observed by Pandey JK *etal*, whereas Jaber MH *etal* reported a high prevalence of 28.75%, in his study.[10, 2]

In our study, urine (50.4 %) was the predominant sample of isolation(Table-1). It is comparable with these studies [11 & 12.]

Proteus is one of the important organism in causing UTI. Its urease enzyme cause polyvalent cations such as Mg+, ca + which will precipitate the urine and form struvite stones causes obstruction of the urinary tract, leads to persistence of the bacterium and makes the treatment difficult. *P.mirabilis* has a higher propensity for colonizing the urinary tract than *P.vulgaris & P.penneri*, due to the difference in its pathogenicity [1]

In this study, *Proteus* spp were equally isolated (44.6%) from pus (Table-1). Feglo *et al.*, [13] and Leulmi *et al*[14] observed maximum isolates in pus samples.

Male (63.6%) were commonly affected than female (36.4%)(Table-2). UTI due to *Proteus* spp is more common in males. It is concordant with the studies of Bahashwan *et al* & Nita Pal *et al.* [9,1] Male prepondarence is mostly due to frequent increased outdoor activities and exposure to environment and infectious agents.

In our study, 3 *Proteus* species *P.mirabilis, P.vulgaris & P.penneri* were isolated at the rate of 66.9%, 31.5% & 1.6% respectively. (Figure-1). *P.mirabilis* was the predominant isolates in variuos reports. [6, 10, 14, 15, 16.] *P.mirabilis* causes 90% of Proteus infections such as meningitis,empyema, osteomyelitis and gasteroenteritis. Also, it is implicated in nosocomial infections of urinary tract(46%),surgical wounds(24%) and respiratory tract (30%). It is believed that the most common cause of infections related to kidney stone, is the most

common serious complication of unresolved and recurrent bacteriuria. So early isolation and speciation of *Proteus* and monitoring the antibiogram pattern will help to prevent the complications.

Imipenem was the most effective drug of resistant strains of gram negative bacilli.[17] In our study, results of antimicrobial susceptibility test revealed that Imipenem was the most effective antibiotic against *Proteus* spp with the sensitivity rate of 99.1%, which is followed by Piperacillin-Tazobactam with the sensitivity rate of 92.5%.(Table-3). Similar pattern were observed with Nita Pal *et al*. & Shenoy *et al.* [1,18]. Though imipenum was found to be unaffected by the enzymes in our study, the variation in the resistance reports could be due to the study environment.

The *Proteus* spp in our study were moderatly sensitive to ofloxacin, amikacin and ciprofloxacin, which were 61.1%, 55.4% & 51.2% respectively. In Proteus infections, moderately sensitive drugs can be used as synergic combined antimicrobial therapy to avoid the imipenum resistance which can be kept as reserve drug.

All the isolates showed lower sensitivity rate of 19.8 % & 8.26% to Amoxyclav & Ampicillin respectively. It is in accordance with the studies of Nita Pal *et al* & Vinoth *et al*., [1,16]

Attention should be focused on the decreasing trend of susceptibility to this group of drugs because prescription of these antibiotics to *Proteus* infections will end up with multi drug resistance (MDR) , extended drug resistance (XDR) and pan drug resistance (PDR) which is worrisome. MDR is pervasive and emerging clinical problem, which causes significant morbidity, mortality and increased economical burden which stems from the inappropriate, excessive use of antibiotics.

The antibiotic resistant pattern of *Proteus* may be an indication of the resistant levels among the Enterobacteriaceae and provides selective pressure, may lead to higher level prevalence of resistant bacteria and could serve as potential reservoir of resistant genes. [19]

Proteus spp are capable of producing beta lactamase that will hydrolyze beta-lactum drugs. So the *Proteus* isolates has to be screened for beta lactamase. The frequency of ESBL producing strains in our study is 24.8 %.(chart-1). Slightly high rate of isolation, 48.86% was noted with Pandey JK *et al*. [10], and a very high rate of 69.44% of *Proteus* was ESBL producers in urinary isolates in the study of Nachimuthu Ramesh *et al*. [20]

The ESBL resistance patterns of the isolates are indicators of an increase in the resistance menace reported by earlier studies. [3]

Emergence of infections caused by ESBL, MBL, MDR, XDR & PDR *Proteus* is alarming which creates serious health problem resulting in an enormous burden in health care setup and cost.

Reducing susceptibility of *Proteus* spp and the emerging resistance illustrated in this group need for routine susceptibility tests. This study is therefore a step towards the generation of national data on the prevalence of antimicrobial resistance pathogens.

Limitations of this study

Limited documentation of research works are available about the prevalence of *Proteus* resistance and paucity of large scale molecular study to know the resistant gene prevalent in our area to formulate antibiotic policy.

CONCLUSION

Awareness about the resistance among these species is of concern because they are the potential reservoir of resistant genes that could be transferred to other bacterial pathogen. The raising resistance to various group of antibiotics is a common problem and its management is a subject of concern. Species identification, surveillance and study of the epidemiology of antimicrobial resistance will assist in the therapeutic management of patients by reducing the prescription of large spectrum antibiotics control of infections.

Table-1
Distribution of *Proteus* spp among various specimen

Specimens	Number (n=121)	Percentage (%)
Urine	61	50.4
Pus	54	44.6
Blood	3	2.5
Sputum	2	1.7
Peritoneal fluid	1	0.8

Table-2
Sex distribution of *Proteus* spp

Sex	Number (n=121)	Percentage (%)
Male	77	63.6
Female	44	36.4

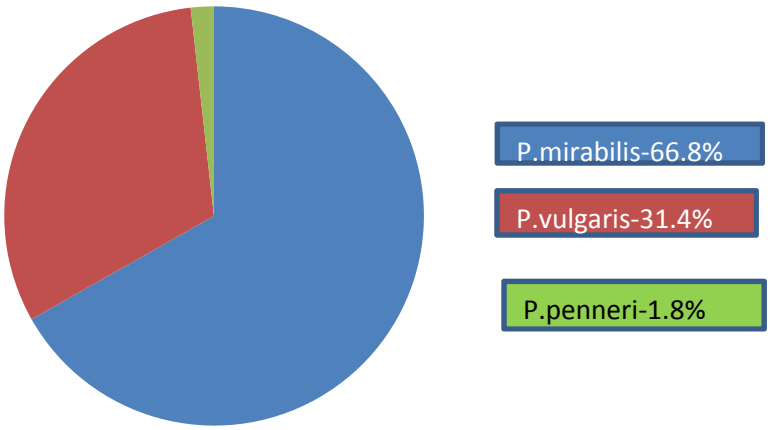

Figure-1
SPECIES DISTRIBUTION (n=121)

Table-3
Antibiotic susceptibility pattern of *Proteus* spp.

Antibiotics	Number (n=121)	Percentage (%)
Imipenem	120	99.1
Piperacillin tazobactum	112	92.5
Ofloxacin	74	61.1
Amikacin	57	47.1
Ciprofloxacin	52	42.9
Cefuroxime	45	37.1
Ceftriaxone	41	33.8
Doxycycline	35	28.9
Gentamycin	35	28.9
Ceftazidime	32	26.4
Amoxyclav	24	19.8
Ampicillin	10	8.26

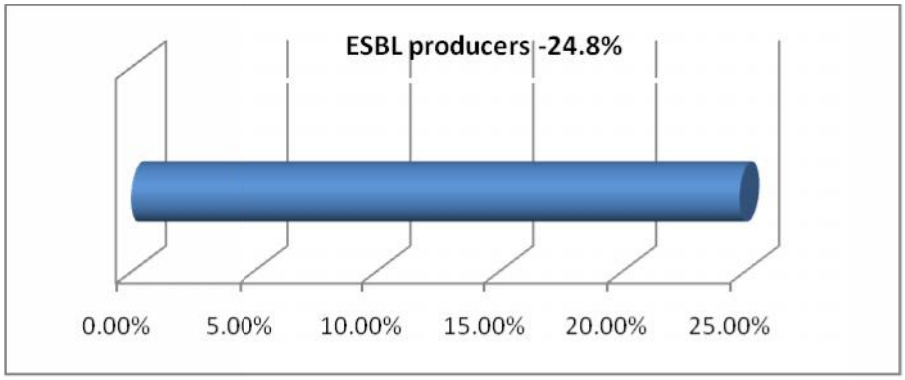

Chart-1
ESBL DISTRIBUTION

REFERENCES

1. N Pal, N Sharma, R Sharma, S Hooja, and RK Maheshwari. Prevalence of Multidrug (MDR) and Extensively Drug Resistant (XDR) Proteus species in a tertiary care hospital. India, Int.J.Curr.Microbiol.App.Sci. 2014; 3 (10): 243-252.
2. Jabur MH, Saedi EAL, Trad JK. Isolation of Proteus mirabilis and Proteus vulgaris from different clinical sources and study of some virulence factors from Babylon University, College of medicine. Pure and Applied Sciences.2013;21(1):43-48.
3. Newman MJ,Frimpong E, Asamoah-Adu A and Sampane-Donker,Resistance to Antimicrbial Drugs in Ghana. The Ghanaian Dutch collaboration for Health Research and Development. 2006;1-6.
4. Enbulele O,Yah SC, Yusuf EO and Eghafona NO,Emerging quinolones resistant transfer genes among Gram-negative bacteria isolated from feces of HIV/AIDS patient attending some clinics and hospitals in the city of Benin ,Edo state , Nigeria . online health 1762 Afr . J. Biotechnol L. Allied Scs. (2006). 3:3.
5. Warren JW, Tenney JH and Hoopes JM.A prospective microbiologic study of bacteriuria in patients with chronic indwelling ureteral catheters . J.Infect. Dis. (1992) ;146:719-723.
6. Mishra M , Thakar YS and Pathak AA. Haemagglutination , haemolysin production and serum resistance of Proteus and related species isolated from clinical source . Indian . J.Med .Microbiol.2001: 19 : 5-11.
7. Koneman EW, Allen SD ,Janda WM ,Schreckenberger PC, Winn WC. Color Atlas And Textbook of Diagnostic Microbiology 6th edition, Philadelphia, USA, Lippincott-Raven Publishers, 2008.
8. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty third informational supplement. 2013:44-61
9. Bahashwan SA, Shafey HME.Antimicrobial Resistance Patterns of Proteus Isolates from Clinical Specimens. European Scientific Journal 2013;9(27):188-202.
10. Pandey JK, Tyagi AKS. Prevalence of Proteus species in clinical samples, antibiotic sensitivity pattern and ESBL production. Int.J.Curr.Microbiol. App.Sci. 2013; 2(10):253-61.
11. Orett FA,Prevalence of Proteus species in urinary tract infections in a regional hospital in Trinidad. Zhonghua Yi Xue Za Zhi (Taipei). 1999; 62:438-442.
12. Reslinski A,Gospodarek E and Mikucka A, Prevalence of multi-drug resistant Proteus species in clinical specimens and their susceptibility to antibiotics. Med.Dosw. Micribial. 2005; 57(2):175-184.
13. Feglo PK, Gbedema SY, Quay SNA, Adu-Sarkodie Y, and C. Opoku-Okrah. Occurrence, species distribution and antibiotic resistance of Proteus isolates: A case study at the Komfo Anokye Teaching Hospital (KATH) in Ghana. International Journal of Pharma Sciences and Research (IJPSR). 2010;1(9): 347-352
14. Leulmi Z, Kandouli C, Benlabed K,Lezzar A, Ilhem Mihoubi I. Prevalence and evaluation of resistance to antibiotics of genera Proteus, Morganella and Providencia isolates in University Hospital of Constantine, Algeria. International Journal of Advanced Research. 2014;2(1):220-27.
15. Jones R, Bacteraemia,England, Wales and Northern Ireland:Commun Dis Rep CDR. Wkly [serialonline] 2003.

16. J Vinoth, ES Begum, RS Kumar and S Ramesh, Phenotypic detection and antibiogram of ampc beta – lactamases producing tribe proteeae in a tertiary care hospital. Asian Journal of Pharmaceutical and clinical Research. 2012:5(4);180-182
17. El-Tahawy A.T (2000). Bacteriology of diabetic foot infections. Saudi Medical Journal. 21 (4): 344-347.
18. Shenoy SM, Mohit, Sinha R.Antibiotic sensitivity pattern of clinical isolates of Proteus species with special reference to ESBL and Amp C production. Indian Journal of Advanced Research. 2013;3(3):293-4
19. Levy. S.B., 1999. Antibiotic resistance: anecological inbalance. Ciba Found.Symp. 207:1-14.
20. Nachimuthu Ramesh, Chettipalayam Samiappan Sumathi, Velraman Balasubramanian, Kurumandur Ravichandren Palaniappan,Velu Rajesh Palaniappan; urinary tract infection and antimicrobial susceptibility pattern of ESBL producing clinical isolates. Advances in Biological Research 2(5-6);78-82,2008.