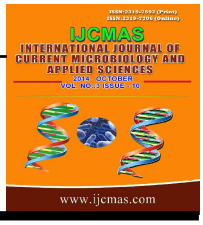
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 243-252**
http://www.ijcmas.com



Original Research Article

Prevalence of Multidrug (MDR) and Extensively Drug Resistant (XDR) *Proteus* species in a tertiary care hospital, India

Nita Pal*, Nikita Sharma, Rajni Sharma, Saroj Hooja, and Rakesh K Maheshwari

Department of Microbiology, SMS Medical College, Jaipur (Rajasthan), India

**Corresponding author*

Keywords

Proteus, multiple drug resistance, extensively drug-resistance

A B S T R A C T

Different *Proteus* species are among the commonly implicated pathogens in hospital as well as community acquired infections. They can cause infection in different anatomical sites of the body due to different modes of transmission. To determine the prevalence of different *Proteus s*pecies and antibiotic resistance pattern. Isolation and identification of proteus species was done by conventional microbiological procedures. Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method. One hundred one *Proteus* species were isolated from 3922 clinical specimens, giving prevalence of 5.38 %. Wound isolates were the highest (67.85%) followed by urine (19.64%). Three *Proteus* species were isolated; *P. mirabilis* (62.37 %), *P. vulgaris* (29.70%) and *P. penneri* (7.92 %). Males were found to be more vulnerable than females in acquiring *Proteus* infections. Among the in-patients infection rate was 81.18% and out-patients 18.82 %.All the species were resistant amoxicillin-clavulanic acid, ceftazidime, cefepime, amikacin, gentamycin and netilmycin. Piperacillin-tazobactam and imipenem were the most active antibiotics followed by meropenem. *P. penneri* was the most resistant species. More than 95% of all isolates were found to be multiple drug resistant (MDR) and more than 50% were possible extensively drug-resistant (XDR). None of the isolates were pan drug-resistant (PDR).

Introduction

The genus *Proteus* along with genus *Providencia* and *Morganella* belongs to the tribe Proteeae of the family Enterobacteriaceae. The genus *Proteus* currently consists of four named species (*P.mirabilis*, *P.penneri*, *P.vulgaris*, *P.myxofaciens*) and four unnamed genomospecies (3, 4, 5, and 6). Genomospecies 3 was named *Proteus hauseri* to honor Hauser who first described the genus. [13]

Both *P. mirabilis* and *P. vulgaris* are widely distributed in the environment with reservoirs in soil, water, sewage and feces and have been isolated from the intestinal tract of mammals, birds and reptiles. *P. penneri* is absent in the intestines of livestock.

Proteus species (spp.) are 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. The modes of transmission may include nosocomial sources, such as hospital food and equipment, intravenous solutions and human contact through contaminated skin surfaces. *Proteus* spp. (*P. mirabilis, P. vulgaris*, and *P. penneri*) are important pathogens of the urinary tract and the primary infectious agents in patients with long-term indwelling urinary catheters. *P. mirabilis* has additionally been implicated in bacteremia, neonatal meningoencephalitis, empyema, formation of calculi and osteomyelitis. *Proteus penneri* has been isolated from a number of diverse clinical sites, including abdominal wounds, urine, bladder calculi, epidural ulcers, bronchoalveolar lavage fluid, stool and infected conjunctiva. Investigations on characterization or pathogenicity of *P. myxofaciens* in the human host have not been reported. [13]

The phenomenal evolution and increase of multidrug-resistance of many bacterial pathogens is increasing and representing a growing public health problem in the world. Multidrug-resistance of *Proteus* spp. calls for regular review of antimicrobial sensitivity pattern in order to institute appropriate antibiotic therapy.

This study seeks to determine the prevalence of the various *Proteus* infections and their antibiotic resistance pattern.

Materials and Methods

Specimen collection

A total of 3922 clinical specimens obtained from suspected cases of bacterial infection received in the department of Microbiology, SMS Medical College, Jaipur, India between February and April 2014 were included in the study. Various clinical specimens received were sputum, pus, urine, cerebrospinal fluid (CSF), tracheal swab, endotracheal (ET) aspirate, catheter tip, blood, ear swab, vaginal swab, body fluids and tissues. Demographic data (such as age, sex, in-patient, out-patient status) of the patients was recorded.

Culture and Identification

The clinical specimens were collected aseptically and processed by standard microbiological methods. [9] Colonies that were non-lactose fermenting on MacConkey agar and showed swarming on Blood agar were isolated and identified by biochemical tests based on whether they were positive for phenylalanine deaminases production; H_2S gas production; and urease reactions. *P. vulgaris* produces indole which differentiated it from indole negative *P.mirabilis* and *P.penneri.* Maltose fermentation and lack of ornithine decarboxylase differentiated *P.penneri* from *P.mirabilis*. [9, 13]

Antimicrobial susceptibility test

Kirby-Bauer disk diffusion method was used to test the susceptibility of the *Proteus* isolates to different antimicrobial agents [obtained from Hi-Media, Mumbai, India] : Amoxicillin-Clavulanic acid (20/10 µg), Piperacillin-Tazobactam (100/10 µg), Cefotaxime (30µg), Ceftazidime (30µg), Cefuroxime (30µg), Cefepime (30µg), Cefoxitin (30µg), Amikacin (30µg), Cotrimox(25µg), Gentamycin(10µg), Ciprofloxacin(5µg), Netilmycin (30µg), Imipenem (10µg), Meropenem (10µg), Doxycycline (30µg). *E. coli* ATCC 25922 was used as the control and the results were interpreted as per Clinical Laboratory

Standards Institute (CLSI) criteria. [3]

Results and Discussion

A total of 3922 clinical specimens were received for bacteriological culture & antimicrobial susceptibility testing. Microorganisms were isolated in 1876 specimens out of which 101 (5.38 %) were *Proteus* spp. (Table 1) Majority of the *Proteus* spp. were isolated from pus (80.19%) followed by urine (8.91%), vaginal swabs (2.97%), tissue (2.97%), blood (1.98%), sputum (1.98%), and body fluids (0.99%).

Three *Proteus* species recovered from 101 specimens were *Proteus mirabilis* (62.37%), *Proteus vulgaris* (29.70%), and *Proteus penneri* (7.92%). (Figure 1)

P.mirabilis was the most frequently isolated species(63/101; 62.38%) followed by *P.vulgaris* (30/101;29.70%) and *P.penneri* (8/101;7.92%).The highest prevalence of *Proteus* infection was observed in the age group 30-39 years accounting for 29.70% of the cases followed by 20-29 years age group in which the prevalence was 28.71%.(Table 2)

Table 3 shows the number of *Proteus* species isolated from different specimens obtained from male and female patients. Males were found to be more vulnerable than females in acquiring *Proteus* infections. A total of 75 (74.25%) isolates were from male patients while 26 (25.74%) were from female patients.

Proteus isolates from in-patients was highest from pus accounting for 68 (67.32 %) of the total isolates followed by urinary isolates 7 (6.93%). A total of 51(80.95 %) *P. mirabilis* were isolated from in-patients and 12 (19.04%) from out-patients. *P. vulgaris* was isolated from 23(76.66%) in-patients and 7 (23.33 %) out-patients. All 8(100 %) *P. penneri* isolates were recovered from in-patients. (Table4)

Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method. Isolates were considered non-susceptible to an antimicrobial agent when it tested resistant or intermediate when using clinical breakpoints as interpretive criteria. The isolate was considered as multidrug-resistant (MDR) when non-susceptible to at least 1 agent in more than 3 antimicrobial categories/groups and extensively drug-resistant (XDR) if non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories/ groups i.e. bacterial isolates remain susceptible to only one or two categories. Isolate non-susceptible to all agents in all antimicrobial categories was considered as pan drug-resistant (PDR). *P.vulgaris* and *P.penneri* are intrinsically resistant to non-extended spectrum 2nd generation cephalosporins – Cefuroxime(CTX) and Tetracyclines – Doxycycline (DOX) were not taken into account when applying the definitions for these organisms.[12]

Antibiotic resistance patterns of *Proteus* isolates are shown inTable5 and Figure2. Antibiotics with 90% - 100 % resistance in all three species were amoxicillin-clavulanic acid, ceftazidime and netilmycin. *P. penneri* isolates exhibited a high resistance to most of the antibiotics followed by *P.mirabilis*. More than 90% of all isolates were found to be multiple drug resistant (MDR) and more than 50% were possible XDR (Table 6, Figure 3). None of the isolates were pan drug-resistant (PDR).

Proteus spp. is widespread in the environment and makes a part of the normal flora of the human gastrointestinal tract.

Although *Escherichia coli* accounts for the largest percentage of cases of uncomplicated cystitis, pyelonephritis, and prostatitis, *Proteus* ranks third as the cause of these infections, particularly in hospital-acquired cases.[2]

In the present study *Proteus* was isolated from 5.38% specimens. Similar prevalence has been reported by Feglo PK et al. (2010)[5] and Leulmi Z et al.(2014) [10] *Proteus* infection in other studies ranged from a lower prevalence of 1.12% & 3.0% reported by Pandey JK et al. (2013)[14] and Bahashwan SA et al.(2013)[2] to a high prevalence of 28.75%,15% and 14.4% reported by Al-Bassam WW et al.(2013) [1], Jabur MH et al.(2013) [6] & Kamga HLF et al (2012)[7] respectively.

In our study the highest percentage of *Proteus* was isolated from pus specimens (80.19%) followed by urine (8.91%). Bahashwan SA et al.(2013)[2], Feglo PK et al.(2010)[5], Leulmi Z et al.(2014)[10] & Shenoy SM et al.(2013)[18] reported maximum isolates from pus whereas some studies have reported isolates more commonly from urine than other clinical specimens.[1,4,6,7, 15,17,]

Proteus plays an important role in urinary tract infection (UTI) especially when there are predisposing factors like surgery or catheterization. The virulence factors like adhesion factors, flagella, IgA protease, urease enzyme help the organism to establish an infection. The formation of bladder or kidney stones due to activity of a urease enzyme which causes polyvalent cations, such as $Mg_2^{+3}$and $Ca^{2+}$to precipitate out of the urine and form struvite stones obstructing the urinary tract or the catheters which makes the treatment difficult and also persistence of the bacterium. UTI due to *Proteus* is more common in males whereas infections by *E.coli* are more common in female patients. [18] Similarly in our study most of the isolates were from male patients (66.70 %) with UTI. Similar sex distribution was observed in various studies [2, 10, 14, 18] but Feglo PK et al.(2010) & De Champs C et al.(2000) found no difference.

In the present study three *Proteus* species (*P.mirabilis, P. vulgaris and P. penneri*) were identified. *P. mirabilis* was the most common (62.37 %) among all the isolates which is similar to findings of other studies. [5, 7, 10, 14] In urine specimens majority of the isolates were *P. mirabilis* which has a higher propensity for colonizing the urinary tract due to difference in its pathogenicity. [5]

Indole negative *Proteus* species are invariably incorrectly identified as *P. mirabilis,* missing isolates of *P.penneri* which may be non-swarming on first isolation. All *P. penneri* isolates were isolated from pus specimens similar to other studies [5,14] whereas Kamga HLF et al. (2012) isolated 3% all of which were isolated from urine specimens.

Proteus express virulence factors associated with adherence, motility, immunoavoidance, nutrient acquisition, host damage, biofilm formation and endotoxicity and therefore behave as opportunistic pathogens in nosocomial infections. [16] We found majority of *Proteus* infections among the in-patients (81.18%) as compared to out-patients (18.82 %) as in other studies. [4, 5, 10, 14] All strains of *P. penneri* were isolated from in-patients.

All the three *Proteus* species displayed high antimicrobial resistance rates to amoxicillin-clavulanic acid, ceftazidime, cefepime, amikacin, gentamycin and netilmycin. Resistance to carbapenems was 12.50%. High antimicrobial resistance was also

observed in many studies. [1, 2, 14, 15, 17, 18, 19] None of our isolates were resistant to antipseudomonal penicillins+ β-lactamase inhibitors (piperacillin-tazobactam) while Shenoy SM et al. (2013) reported a low resistance of 8.3%. We found more than 90% of all isolates to be multi-drug resistant (MDR). MDR *Proteus* reported by Feglo PK et al.(2010) was 88%, Leulmi Z et al.(2014) 61%, Pandey JK et al. (2013) 48.86% and Tumbarello M et al. (2012) 36 % (in blood stream infections). In our study more than 50% were possible XDR and none were PDR. The high prevalence of MDR & XDR isolates may be due to the fact that most of the specimens were received from in-patients. A review of the antimicrobial resistance profile of isolates showed that piperacillin-tazobactam (PIT) and imipenem (IMP) were the most active antibiotics followed by meropenem (MRP).

P.penneri has the ability to cause major infectious diseases and nosocomial outbreaks and carries similar pathogenic determinants to *P. mirabilis* and *P. vulgaris*. [8] All isolates of *P.penneri* were isolated from pus specimen of in-patients and exhibited multi-drug resistance and 75% were possible XDR. Feglo PK et al. (2010), Kishore J (2012) and Pandey JK et al.(2013) also reported high MDR in *P. penneri.*

The high antibiotic resistance of *Proteus* may be an indication of the resistance levels among the Enterobacteriaceae since indiscriminate use of antibiotics provides selective pressure, leading to a higher prevalence of resistant bacteria which is very common in developing countries. [11]These species are potential causes of infections and reservoirs of resistance genes that could be transferred to other bacterial pathogens.

Table1. Distribution of *Proteus* isolates from various clinical specimens

Types of specimen	No. of samples	No. of organism isolated	No. of Proteus isolated (%)
Urine	1320	555	9 (1.62)
Pus	570	505	81 (16.03)
Blood	1319	343	2 (0.58)
Endotracheal aspirate(ET)	6	6	0 (0)
Sputum	172	132	2 (1.5)1
Ear swab	4	0	0 (0)
Vaginal swab	36	35	3 (8.57)
Tracheal swab	101	95	0(0)
Catheter tip	36	33	0 (0)
Tissue	13	9	3 (33.3)3
Body fluids	168	103	1(0.97)
Cerebrospinal Fluid (CSF)	177	60	0 (0)
Total	3922	1876	101(5.38)

Figure1. Distribution of various *Proteus* species isolated

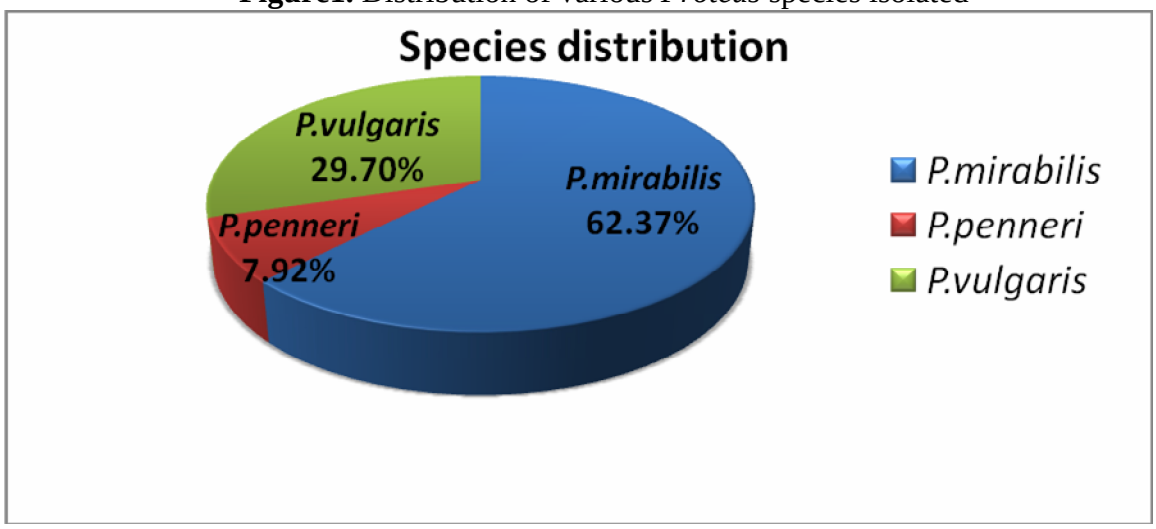


Table2. Distribution of *Proteus* species in different age groups

Age group (years)	*Proteus mirabilis*	*Proteus vulgaris*	*Proteus penneri*	Total (%)
0-9	3	0	0	3 (2.97)
10-19	7	3	1	11 (10.89)
20-29	22	6	1	29 (28.71)
30-39	18	10	2	30 (29.70)
40-49	10	5	1	16 (15.84)
50-59	1	4	3	8 (7.92)
>60	2	2	0	4 (3.96)
Total%	63(62.38%)	30(29.70%)	8(7.92%)	101

Table3. Sex-wise distribution of *Proteus* species isolated from clinical specimens

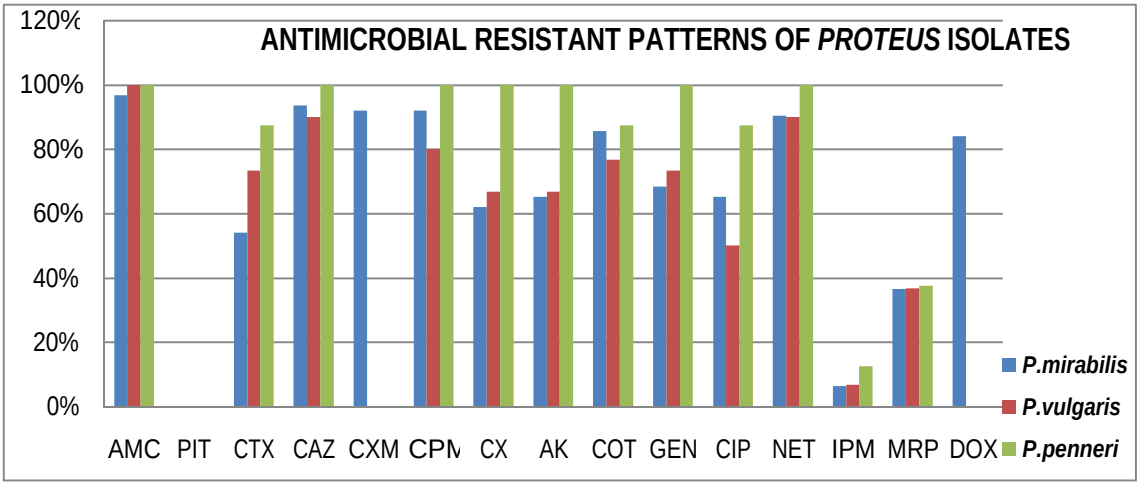
Types of specimen	Male	Female	Total no. of *Proteus* isolated
Pus	62	19	81
Urine	6	3	9
Blood	1	1	2
Sputum	2	0	2
Vaginal swab	-	3	3
Tissue	3	0	3
Body fluids	1	0	1
Total	75	26	101

Table4. Distribution of *Proteus* isolates among in- and out-patients

Samples (No. studied)	Proteus spp.	In-patients	Out-patients	Total no. of species
PUS	Pm	42	7	49
(81)	Pv	18	6	24
	Pp	8	0	8
URINE	Pm	7	1	8
(9)	Pv	1	0	1
	Pp	0	0	0
BLOOD	Pm	0	0	0
(2)	Pv	2	0	2
	Pp	0	0	0
SPUTUM	Pm	2	0	2
(2)	Pv	0	0	0
	Pp	0	0	0
VAGINAL SWAB	Pm	0	2	2
(3)	Pv	0	1	1
	Pp	0	0	0
TISSUE	Pm	0	2	2
(3)	Pv	1	0	1
	Pp	0	0	0
BODY FLUIDS	Pm	0	0	0
(1)	Pv	1	0	1
	Pp	0	0	0
TOTAL		**82**	**19**	**101**

Pm = *Proteus mirabilis*; Pv = *Proteus vulgaris*; Pp = *Proteus penneri*

Figure.2 Antimicrobial resistant patterns of *Proteus* isolates



AMC:Amoxicillin-Clavulanic acid; PIT: Piperacillin-Tazobactam; CTX: Cefotaxime;CAZ: Ceftazidime; CXM: Cefuroxime; CPM: Cefepime, CX: Cefoxitin; AK:Amikacin ;COT :Cotrimox; GEN :Gentamycin; CIP: Ciprofloxacin; NET: Netilmycin ;IPM: Imipenem; MRP: Meropenem; DOX: Doxycycline.

Table5. Percentage of antimicrobial resistance of *Proteus* isolates

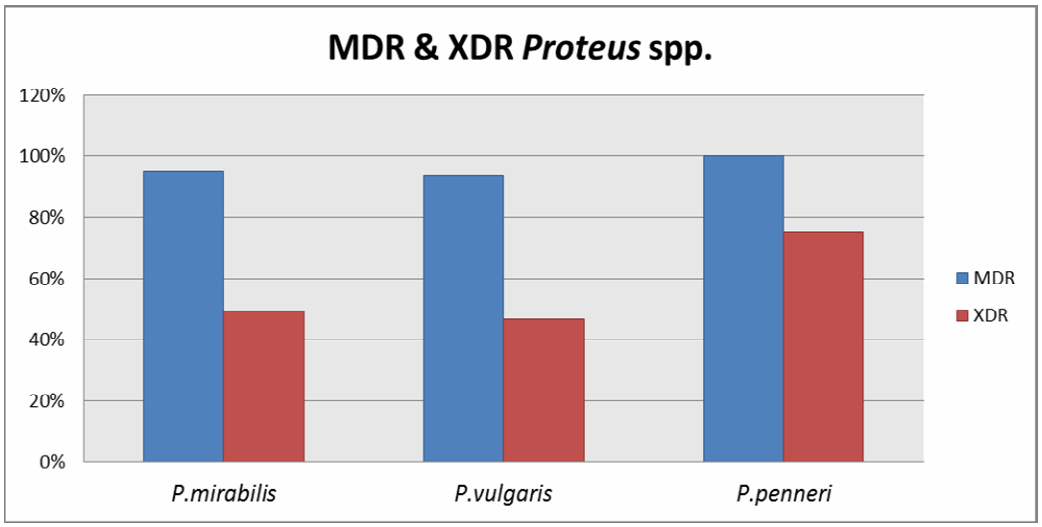
Antibiotic	Percentage of antibiotic resistance		
	P.mirabilis	*P.vulgaris*	*P.penneri*
Amoxicillin-Clavulanic acid	97%	100%	100%
Piperacillin-Tazobactam	0%	0%	0%
Cefotaxime	54%	73.33%	87.50%
Ceftazidime	94%	90%	100%
Cefuroxime	92%	*	*
Cefepime	92%	80%	100%
Cefoxitin	62%	66.67%	100%
Amikacin	65%	66.67%	100%
Cotrimox	86%	76.67%	87.50%
Gentamycin	68%	73.33%	100%
Ciprofloxacin	65%	50%	87.50%
Netilmycin	90%	90%	100%
Imipenem	6%	6.67%	12.50%
Meropenem	37%	36.67%	37.50%
Doxycycline	84%	*	*

* Intrinsic resistance

Table 6. Multi-drug resistant (MDR) & possible Extensively drug-resistant (XDR) *Proteus* isolates

Organisms isolated	Total no. of isolates	No. of isolates found to be	
		MDR (%)	Possible XDR (%)
P.mirabilis	63	60 (95.24)	31(49.20)
P.vulgaris	30	28 (93.33)	14(46.66)
P.penneri	8	8 (100)	6(75.00)
Total	101	96 (95.05)	51 (50.49)

Figure3. Distribution of MDR and XDR among *Proteus* species



The presence of *Proteus* in clinical specimens is of great importance since like other Enterobacteriaceae they are opportunistic pathogens and may cause morbidity and mortality. There was variability in the frequency of isolation of *Proteus* from clinical specimens. In this study, majority of the *Proteus* isolates were obtained from wound (80.19%).

Three *Proteus* species recovered were *P.mirabilis* (62.37%), *P.vulgaris* (29.70%), and *P.penneri* (7.92%). Epidemiological patterns of *Proteus* infections in the service areas showed that most isolates were from in-patients (81.18%). A high prevalence of *Proteus* infections was probably due to the prolonged hospitalization, overcrowding in the wards and indiscriminate use of antibiotics. All isolates from this study exhibited resistance to antibiotics commonly used in hospitals. More than 90% of all isolates were found to be MDR and > 50% possible XDR may be due to our institution being a tertiary care referral institute and most of the specimens were received from in-patients where prevalence of nosocomial infection is high. However, they were susceptible to piperacillin-tazobactam, imipenem & meropenem which are the options left for treatment of *Proteus* infections. *P.penneri* showed high antibiotic resistance as compared with other isolates.

Resistance to antibiotics is an increasingly common problem and its management is a subject of concern. Species identification and study of the epidemiology of antimicrobial resistance will assist in the management and control of infections. It will also help in the therapeutic management of patients by reducing the prescription of large spectrum antibiotics. Therefore this study is a step towards the generation of data on the prevalence of antimicrobial resistant pathogens in our institution.

Acknowledgements

We will like to acknowledge Laboratory technicians Mr.R.K.Yadav & Mr.Rajkumar Sharma for their assistance in this study.

References

1. Al-Bassam WW, Kazaz AKA. The Isolation and Characterization of *Proteus mirabilis* from Different Clinical Samples.Journal of Biotechnology Research Center 2013;7(2):24-30.
2. Bahashwan SA, Shafey HME. Antimicrobial Resistance Patterns of *Proteus* Isolates from Clinical Specimens. European Scientific Journal 2013;9(27):188-202.
3. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty third informational supplement. 2013:44-61
4. De Champs C, Bonnet R, Sirot D, Chanal C and Sirot J. Clinical relevance of *Proteus mirabilis* in hospital patients: A two year survey. J Antimicrob. Chemoth. 2000;45:537-9.
5. Feglo PK, Gbedema SY, Quay SNA, Adu-Sarkodie Y, and Opoku-Okrah C. 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;1:347-52.
6. 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 Sciences2013;21(1):43-48.

7. Kamga HLF, Assob JCN, Nsagha DS, Njunda AL, Nde Fon P., Tchape GNE. Epidemiological studies on Proteeae isolates from clinical specimens in the Laquintinie Hospital in Douala, Cameroon. African Journal of Clinical And Experimental Microbiology AJCEM).2012;13:112-20.
8. Kishore J. Isolation, identification & characterization of *Proteus penneri* - a missed rare pathogen. Indian J Med Res; 2012;135:341-345.
9. Koneman EW, Allen SD ,Janda WM , Schreckenberger PC, Winn WC. Color Atlas And Textbook of Diagnostic Microbiology 5th edition, Philadelphia,USA,Lippincott-Raven Publishers, 1997.
10. 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.
11. Levy SB. Antibiotic resistance: an ecological inbalance. In Ciba Found.Symp. (Ed.)1999;207:1-14.
12. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME,Giske CG, et al Multidrug-resistant, extensively drug-resistant and pandrug-resistantbacteria: an international expert proposal for interim standard definitions for acquired resistance.European society of Clinical Microbiology and Infectious diseases2012;18: 268–281.
13. O'Hara CM, Brenner FW and Miller JM. Classification, identification, and clinical significance of *Proteus, Providencia,* and *Morganella*. Clin.Microbiol.Rev. 2000;13: 534-46.
14. 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.
15. Reslinski A, Gospodarek E, Mikucha A. Prevalence of multi-drug resistant *Proteus species* in clinical specimens and their susceptibility to antibiotics.Med. Dosw. Micribial 2005;57(2):175-84.
16. Rozalski A, Torzewska A, Moryl M, Kwil I, Maszewska A, Ostrowska K et al. *Proteus* sp. – an opportunistic bacterial pathogen – classification, swarming growth, clinical significance and virulence factors. *Folia Biologica et Oecologica* 2012;8:1–17.
17. Sekowska A, Janicka G., Wróblewska J, and Kruszyńska E (2004). Prevalence of *Proteus mirabilis* strains in clinical specimens and evaluation of their resistance to selected antibiotics. Pol MerkurLekarski. 2004;17:538-40.
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. Tumbarello M, Trecarichi EM, Fiori B, Losito AR, D'Inzeo T, Campana L, et al. Multidrug-Resistant *Proteus mirabilis* Bloodstream Infections: Risk factors and outcomes. Antimicrob. Agents Chemoth. 2012;56(6):3224-31.