DOI: 10.7860/JCDR/2022/51928.15901

Original Article

Microbiology Section

Phenotypic Characterisation of *Proteus* Species Isolated from Different Clinical Samples with Special Reference to Antibiotic Resistance Pattern in a Tertiary Care Centre

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ABSTRACT

Introduction: The *Proteus* species are vulnerable to cause community-acquired and Healthcare Acquired Infections (HCAI). This organism is grouped under opportunistic pathogen and implicates a wide range of infection in humans. The emergence of antimicrobial resistance in this species is alarming and life threatening.

Aim: To evaluate the prevalence and the resistance pattern of *Proteus* species isolated from various clinical samples by conventional culture methods.

Materials and Methods: This was a cross-sectional study conducted from May 2020 to April 2021. The study was conducted at SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India, after approval from the Institutional Ethical Committee. *Proteus* isolates from various clinical samples like wound swab, pus, urine, Cerebrospinal Fluid (CSF), tracheal swabs, endotracheal aspirate, vaginal swabs, blood, body fluids, ear swab, tissue were collected as per standard protocols. The phenotypic characterisation and resistance pattern of *Proteus* isolates were done by conventional culture methods. The analysis was done using Statistical Package for the Social Sciences (SPSS) software version 24.0.

Results: Out of 100 isolates, the higher number of *Proteus* species was isolated from pus sample (35%), wound swab (22%) and urine sample (25%). Males were found to have a higher prevalence (65%) of infections by *Proteus* species than females. *Proteus mirabilis* was the most common isolated species (89%) in this study. The Extended Spectrum Beta Lactamase (ESBL) producers among *Proteus* species were found to be 39%, AmpC producers to be 20% and carbapenemase producer 1%. *Proteus* species had the highest sensitivity to piperacillin tazobactam (100%), tigecycline (100%), meropenem and imipenem (98%) in this study.

Conclusion: It was concluded that there was an increasing occurrence of drug resistance *Proteus* species. Their resistance is contributed by production of ESBL, AmpC and carbapenemase enzymes. The screening test alone is sufficient for detection of antibiotic resistance. Hence, there is a need for reliable phenotypic confirmatory test to identify the resistance among *Proteus* species.

Keywords: Antimicrobial resistance, Non lactose fermenter, *Proteus mirabilis*, *Proteus vulgaris*, Swarming

INTRODUCTION

The *Proteus* genus is included in tribe Proteeae comes under the family of Enterobacteriaceae which are pleomorphic gram negative bacilli. The *Proteus* species are mostly saprophytic which can be isolated commonly from sewage and decomposing animal matter [1]. In concordance to humans, this species is a commensal of skin and intestine. They are grouped under opportunistic pathogen that causes infections mainly in urinary tract, skin and soft tissues [2]. The main pathogenic organism in this family includes *Proteus mirabilis* (*P. mirabilis*), *P. vulgaris* and *P. penneri*.

Among these species, the most commonly isolated species is *P. mirabilis* which causes Urinary Tract Infection (UTI) and wound infection. In addition, *P. mirabilis* is also found to be the cause of empyema, bacteraemia, neonatal meningoencephalitis, renal calculi, diarrhoea and osteomyelitis. *P. vulgaris* is mostly isolated from patients who are immunocompromised like people who are on long term antibiotics [1,2]. *Proteus* species corresponds to 10-15% of complicated UTI in catheterised patients. *P. mirabilis* is considered as the most commonly encountered pathogen causing UTI. The prevalence of UTI in chronically catheterised patients by *Proteus* is about 20-45% [3].

P. penneri is capable of outbreaks of nosocomial infections [4]. Few articles have documented that *P. penneri* can be isolated from the patients suffering from UTI, wound and epidural ulcers and samples such as pus, conjunctiva, bronchoalveolar lavage [5]. It is isolated from the urine at a prevalence of 50%, Skin and Soft tissue Infection (SSI) with 25% and blood samples at 15% [6]. The *Proteus* species is also found to cause UTI in patients suffering from urinary tract abnormalities and catheterised patients. The *Proteus* infection is found to be the reason for the development of renal stones and pyelonephritis [6]. The virulence factors of *Proteus* species is varied and stated to be the production of urease, swarming, formation of biofilm, formation of calculi, proteolytic enzymes, fimbriae and haemolysin production [7,8].

The *Proteus* species are also found to be infecting diabetic patients at a higher rate. They can cause tissue destruction in these patients [9]. They are opportunistic pathogens and the main source of transmission includes equipment's used in hospital, open wounds, trauma and patient to patient spread through contaminated surfaces [4]. The Multidrug-Resistant (MDR) *Proteus* species is increasing in health sectors. The increase in resistance to antibiotics accounts to challenge in the treatment of patient which increases the death rate. The mechanism by which *Proteus* species shows resistance to antibiotics includes the production of ESBL, carbapenamase and AmpC [10].

The majority of resistance is caused by ESBLs which is a major group of beta-lactamases [11]. The ESBL shows resistance to ceftazidime but sensitivity to ceftazidime in combination with clavulanic acid. The ESBL production shows a high risk for the community and accounts for many outbreaks. The major threats posed by ESBLs include ineffectiveness of antibiotics, high cost, increased hospital stay and increased complications. The treatments are becoming limited

due to the rapid increase in the ESBL. The screening of ESBLs is required to control the infection worldwide [12-14]. Another major class, AmpC beta-lactamases cause resistance to the penicillin group of drugs, cephalosporins and monobactams [15]. The next important resistance mechanism is resistance to carbapenems. Carbapenemase resistance poses a major difficulty in treating the severely ill patients. The evolvement of MDR implies major threat to therapeutics and has become the major source of HCAI [16]. The antimicrobial resistance is becoming the main burden to healthcare system. The current study assesses the prevalence of infections caused by *Proteus* species including the resistance pattern which is helpful in characterisation of the *Proteus* species as there is less data available regarding the resistance in *Proteus* species.

MATERIALS AND METHODS

The present study was a descriptive, cross-sectional study done between May 2020 to April 2021. The present study was approved by IEC of SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India (Ethical clearance number: 1934/IEC/2020).

Inclusion criteria: All non duplicate *Proteus* isolates from various clinical samples like pus, sputum, urine, CSF, tracheal swab, endotracheal aspirate, vaginal swab, blood, body fluids, ear swab, and tissue were included. Samples collected before initiation of antibiotic therapy were included in the study.

Exclusion criteria: Isolates other than *Proteus* species from various clinical samples were excluded from the study.

Sample size calculation: Study sample size approx. 100, $n=4pq/d^2$

(Calculated and approved by Statistician).

p=Prevalence rate=5.82% [17]

p=5.8/100=0.582

q=(100-p)

n=4×0.0582×0.9418/0.0025

=0.2193/0.0025

=87.7

=88- final sample size of 100 was taken

Study Procedure

Identification: Clinical samples were obtained in an aseptic manner and processed using standard microbiological techniques [1]. Conventional culture techniques were used to isolate the *Proteus* species. It produces swarming on blood agar, non lactose fermenting colonies on MacConkey agar. The biochemical tests were used to speciate *Proteus*. It shows positive for phenylalanine deaminase test (PPA), abundant H_2S production with gas in Triple Sugar Iron (TSI) agar, and is a rapid urease producer. Indole is positive in *P. vulgaris* that distinguishes it from *P. mirabilis* which is indole negative. *P. penneri* is distinguished from *P. mirabilis* by maltose fermentation and the absence of ornithine decarboxylase [1,18].

Antibiotic Susceptibility Testing (AST): The assessment of antibiotic susceptibility is carried on Muller Hinton Agar (MHA) by the Kirby Bauer disc diffusion method. The Clinical and Laboratory Standards Institute (CLSI) guidelines for 2020 were used to interpret the zone sizes [19]. The following antibiotic disc were used ampicillin (10 µg), amoxicillin-clavulanate (20 µg/10 µg), ceftazidime (30 µg) cetazidime with clavulanuic acid (30 µg/20 µg), cefipime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), ertapenem (10 µg), meropenem (10 µg), piperacillin tazobactam (100 µg/10 µg), amikacin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), cefoxitin (30 µg), cefazolin (30 µg), gentamicin (10 µg), imipenem (10 mg), tetracycline (30 µg), tigecycline (15 µg), nitrofurantoin (300 µg) (purchased from Himedia, Mumbai, India). The zone size was interpreted after 18-24 hours of incubation at 37°C.

Phenotypic Confirmatory Tests for ESBL, AmpC and Carbapenamase Producers

Double disc diffusion test for detecting ESBL: Two antibiotic discs were used namely ceftazidime (30 µg) and cetazidime with clavulanic acid (CAC) (30 µg/20 µg). The CAC is placed in centre of MHA plate and ceftazidime is placed with a distance about 1.5 cm from each other. The inhibition zone development towards CAC disc after incubating at 37°C for 24 hours indicated the presence of an ESBL positive organism [11,12].

Modified three-dimensional test for AmpC producers: On MHA, lawn culture is put, and a cefoxitin disc of 30 µg is placed in the plate. A sterile surgical blade is used to cut a 3 cm long slit 2-3 mm away from cefoxitin drug disc. A well is done at the other end using a pipette tip. The AmpC beta-lactamase enzyme is obtained by alternate freeze and thawing for 7-8 times before centrifuging at 2500 rpm (revolution per minute) for 15 mins. The AmpC beta-lactamase enzymes were released into the suspending fluid as a result of this method. The prepared well is then filled with a total of 20-30 µL of the supernatant containing the extract. After allowing the enzyme to fill and disperse into the slit for 5-10 minutes, the plates were kept at 37ºC up to 24 hours for incubation. A slight heart shaped zone of lysis towards cefoxitin drug suggested a positive result. In this test, the American Type Culture Collection (ATCC) *Escherichia coli* 25922 was used as the indicator strain [20].

Modified Hodge Test (MHT): In peptone broth ATCC *E. coli* 25922 is grown and adjusted to McFarland standards (0.5). The MHA plate is streaked with a 1/10 dilution. In the centre of the test region, a meropenem 10 µg disc is mounted. The test organism is streaked from edge of discs and kept at 37ºC for 16-24 hours. The carbapenem discs were subjected to quality control in compliance with CLSI guidelines. *Klebsiella pneumoniae* ATCC 1705 was used as the control organism [21,22].

STATISTICAL ANALYSIS

The information thus obtained is entered in MS-excel (Microsoft office professional 13) spread sheet for each isolate and the analysis was done using SPSS software version 24.0.

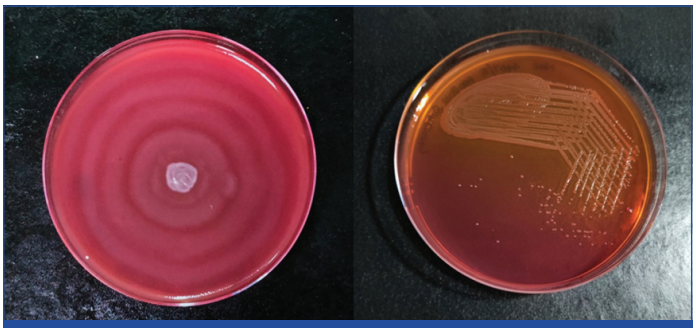
RESULTS

Out of 100 *Proteus* isolates, 39 were isolated between the age group of 61-75 years, 34 were between the age group of 46-60 years and around 15 between the age group of 31-45 year [Table/Fig-1]. *Proteus* isolates were collected, subjected to identification by conventional culture, biochemical and AST. Culture was done on MacConkey agar, Blood agar, and Phenylalanine deaminase test (PPA) were all used to validate the *Proteus* species. In MacConkey agar *Proteus* isolates produced non lactose fermenting colonies. In blood agar *Proteus* species produce a swarming growth which is a unique characteristic feature of this organism [Table/Fig-2]. The PPA test was positive for all *Proteus* species due to the fact that this species is able to produce an enzyme called phenylalanine deaminase that converts phenylalanine to phenylpyruvic acid. The prevalence of *Proteus mirabilis* was found to be 89% whereas *Proteus vulgaris* was found to be 11%. The specimen wise distribution of the

Age (years)	Males	Females	Total
0-15	4	0	4
16-30	2	2	4
31-45	9	6	15
46-60	16	18	34
61-75	30	9	39
>75	4	0	4
Total	65	35	100

[Table/Fig-1]: Demographic distribution.

Proteus species; maximum number of *Proteus* species was isolated from the pus (35%) followed by the urine samples (25%) and wound swab (22%) and other samples as shown in [Table/Fig-3]. The maximum number of *Proteus* species were isolated from wound infection (60%) followed by UTI (20%) [Table/Fig-4].



[Table/Fig-2]: A 24 hour growth showing swarming of *Proteus* species in blood agar (left side) and non lactose fermenting colonies on MacConkey agar (right side).

S. No.	Sample	*Proteus mirabilis*	*Proteus vulgaris*	Total
1	Pus	29	6	35
2	Urine	24	1	25
3	Wound swab	21	1	22
4	Blood	1	0	1
5	Ear swab	3	2	5
6	Tissue	9	1	10
7	Tracheal aspirate	2	0	2
	Total	89	11	100

[Table/Fig-3]: Sample wise distribution shows the specimen wise distribution of the *Proteus* species.

S. No.	Clinical condition	Total number of isolate
1	Wound infection	60
2	Urinary tract infection	20
3	Otitis media	5
4	Kidney abnormalities	5
5	Burn wound	4
6	Bone abnormalities	2
7	Pneumonia	2
8	Sepsis	1
9	Eye infection	1
	Total	100

[Table/Fig-4]: Clinical presentation wise distribution.

Antibiotic susceptibility testing: The AST was performed on the isolates, screening ESBL, AmpC β-lactamase and carbapenamase producers. The zone sizes were interpreted according to CLSI 2020 guidelines. The ESBL producer shows ≥5 differences in zone size between ceftazidime and ceftazidime with clavulanic acid. The resistance to cefoxitin is considered as AmpC producer. The resistance to imipenem, meropenem and ertapenem is considered to be carbapenamase producer. *Proteus* species had the highest sensitivity to piperacillin tazobactam (100%), tigecycline (100%) and meropenem and imipenem (98%) in this study [Table/Fig-5]. The present study demonstrates ampicillin resistance to be 64% and 43% resistance to cefazolin. From the AST report it was found that there were 41 ESBL producer, 25 AmpC producer and two carbapenamase producer.

Confirmatory tests for ESBL, AmpC and Carbapenamase producers:

a. Double disc diffusion test for detecting ESBL: The 41 test isolates which were found to be ESBL producer by standard AST was subjected to phenotypic confirmatory test. The confirmatory test was positive in 39 isolates [Table/Fig-6].

Antibiotics	Resistance percentage
Ampicillin	64%
Amoxicillin-clavulanic acid	48%
Ceftazidime	39%
Ceftazidime with clavulanuic acid	9%
Cefipime	12%
Ceftriaxone	23%
Cefuroxime	38%
Ertapenem	4%
Meropenem	2%
Piperacillin tazobactum	0%
Amikacin	12%
Chloramphenicol	25%
Ciprofloxacin	38%
Cefotaxime	23%
Cefoxitin	24%
Cefazolin	43%
Gentamicin	28%
Imipenem	2%
Tetracycline	33%
Tigecycline	0%

[Table/Fig-5]: Antibiotic resistance pattern of *Proteus* species by disc diffusion method.

S. No.	Resistance mechanism	Screening test	Phenotypic confirmatory test
1	ESBL	41%	39%
2	AmpC	25%	20%
3	Carbapenamase	2%	1%

[Table/Fig-6]: Comparison of screening test and phenotypic confirmatory test for ESBL, AmpC and carbapenamase produce.

b. Modified three-dimensional test for AmpC producers: The 25 test isolates which were found to be AmpC producer by standard AST was subjected to phenotypic confirmatory test. The confirmatory test was positive in 20 isolates [Table/Fig-6].

c. Modified Hodge Test (MHT): The two test isolates which were found to be carbapenamase producer by standard AST was subjected to phenotypic confirmatory test. The confirmatory test was positive in one isolate [Table/Fig-6].

DISCUSSION

Proteus species is found throughout the environment and is a normal part of the human gastrointestinal tract's flora. *Escherichia coli* is considered the most common cause of uncomplicated cystitis, pyelonephritis, and prostatitis. *Proteus* is considered the third most common cause, especially in hospital acquired infections [23]. The maximum numbers of *Proteus* species was between the age group of 61-75 years (39%), 46-60 years (34%) and 31-45 years (15%). Maheswary D and Chitralekha S and Prasad RR et al., found similar results [17,24]. The 89% of the *Proteus* species was found to be *P. mirabilis* in this analysis and 11% was *P. vulgaris*. The variation in prevalence rate between the previous studies may be due to difference in demography and also the *Proteus* is getting highly virulent and spreads at higher rate [Table/Fig-7] [17,25,26].

Since, the antibiotic resistance patterns of *Proteus* species vary with different species hence species reporting is essential. In comparison to *P. vulgaris* that has inherent resistance to variety of antimicrobial agents *P. mirabilis* was found to be the most susceptible species. The highest number of *Proteus* species is collected from the pus sample (35%), followed by the urine samples (25%). *Proteus* species was also isolated from blood (1%), ear swab (5%), tissue (10%) and from tracheal aspirate (2%) sample. Bahashwan SA and

S. No.	Other studies	Prevalence of *Proteus mirabilis* (%)	Prevalence of *Proteus vulgaris* (%)	Others (if any *Proteus penneri*) (%)
1	Nirali D and Dipika P, [25]	88.7	9.13	2
2	Pal N et al., [26]	62.37	29.70	8
3	Maheswary D and Chitralekha S [17]	52.54	30.5	16.94
4	Current study	89	11	-

[Table/Fig-7]: The prevalence of *Proteus mirabilis* and *vulgaris* in comparison to previous studies [17,25,26].

Shafey HME, Feglo PK et al., Leulmi Z et al., and Shenoy SM et al., reported maximum isolates from pus whereas some studies have reported isolates more commonly from urine than other clinical specimens [23,27-32].

When there are predisposing conditions such as surgery or catheterisation, *Proteus* plays a major role in UTI. Adhesion factors, flagella (swarming), Immunoglobulin A (IgA) protease, and the urease enzyme are all virulence factors that assist the organism to establish an infection. The formation of bladder or kidney stones as a result of the activity of the urease enzyme, which causes polyvalent cations such as Mg^{2+} and Ca^{2+} to precipitate out of the urine and form struvite stones, which obstruct the urinary tract or catheters, making treatment difficult and allowing the bacteria to persist and multiply and causing the complications. Males are more likely to have a UTI from *Proteus* [29]. In present study, the majority of isolates (65%) came from male UTI patients with old age group. Various research have shown a similar gender distribution [27,33].

Proteus species had the highest sensitivity to piperacillin tazobactam (100%), tigecycline (100%) and meropenem and imipenem (98%). Kengne M et al., recorded 100% sensitivity for piperacillin tazobactam and meropenem and Preethishree P et al., reported 100% sensitivity for piperacillin tazobactam and meropenem [34,35]. In the present study, amikacin is found to be 88% sensitive whereas gentamicin was found to be 72% sensitive. Alexis A and Sakthivennila M recorded 60% gentamicin resistance [36]. The present study demonstrates ampicillin resistance to be 64%. In comparison to the above analysis, Kargar M et al., reported a high sensitivity percentage of 84% to gentamicin [37]. In contrast to this analysis Kengne M et al., recorded an increase in ampicillin resistance of about 91% [34]. The present study demonstrated the screening test to be 41% ESBL producers and the confirmatory test was positive for 39%. In this present study, AmpC beta lactamases production was detected by screening test in 25% of cases and by confirmatory test of 20% of cases. The carbapenemase producers were found to be 2% in the screening test and 1% in the confirmatory test. Similar results were found in Maheswary D and Chitralekha S [17]. Hence, this study emphasises the data of prevalence along with antimicrobial resistance pattern of *Proteus* species which will provide a good understanding of the resistance pattern at species level and aids in rational use of antibiotics ultimately helping to reduce the increasing antimicrobial resistance threat.

Limitation(s)

The detection of gene responsible for the antimicrobial resistance by molecular methods was not included in the study.

CONCLUSION(S)

There was an increasing occurrence of drug resistance in *Proteus* species. Only screening test will not be sufficient for detection of antibiotic resistance. Hence, reliable phenotypic confirmatory test to identify the resistance among *Proteus* species is required. The confirmatory test such as combined disc diffusion test (ESBL detection), 3-dimensional AmpC assay (AmpC detection) and MHT (Carbapenemase detection) is found to be more significant, reliable and can be carried out easily in a routine microbiology testing laboratories aiding to identify the resistant species and significantly helps in the rationale use of antibiotics.

Acknowledgement

Authors are extremely thankful to SRM Medical College Hospital and Research Centre, Kattankulathur, Chengalpattu district, India for providing us all the equipments and facilities for performing this research work.

REFERENCES

[1] Koneman EW, Koneman AS. Diagnostico Microbiologico/Microbiological diagnosis: Texto Y Atlas En Color/ Text and Color Atlas. in Médica panamericana. 2008;277-79.
[2] Panikar AA, Ananthanarayan R, Ananthanarayan and Paniker's Textbook of Microbiology. Universities Press, Tenth edition. 2005;37:288-89.
[3] Karthik R, Ambica R, Nagarathnamma T. Study of biofilm production and antimicrobial susceptibility pattern in clinical isolates of *Proteus* species at a tertiary care hospital. International Journal of Current Microbiology and Applied Sciences. 2018;7(1):574-86.
[4] Ostwal K, Shah P, Rebecca L, Shaikh N, Inole K. A tale of two novel *Proteus* species-*Proteus hauseri* and *Proteus penneri*. International Journal of Current Microbiology and Applied Sciences. 2016;5(5):84-89.
[5] Krajden S, Fuksa M, Petrea C, Crisp LJ, Penner JL. Expanded clinical spectrum of infections caused by *Proteus penneri*. Journal of Clinical Microbiology. American Society for Microbiology; 1987;25(3):578-79.
[6] Kishore J. Isolation, identification & characterisation of *Proteus penneri*-A missed rare pathogen. Indian J Med Res. 2012;135(3):341-45.
[7] Mishra M, Thakar Y, Pathak A. Haemagglutination, haemolysin production and serum resistance of *Proteus* and related species isolated from clinical sources. Indian Journal of Medical Microbiology. 2001;19(2):05-11.
[8] Syntem S, Dutta H, Kalyani M. Characterisation of *Proteus* species and detection of Multi Drug Resistant (MDR) with special reference to ESBL Strains. International Journal of Current Microbiology and Applied Sciences. 2016;5(11):153-60.
[9] Anandi C, Alaguraja D, Natarajan V, Ramanathan M, Subramaniam CS, Thulasiram M, et al. Bacteriology of diabetic foot lesions. Indian Journal of Medical Microbiology. 2004;22(3):175-78.
[10] Al-Jumaily EFA, Zgaer SH. Multidrug resistant *Proteus mirabilis* isolated from urinary tract infection from different hospitals in Baghdad City. International Journal of Current Microbiology and Applied Science. 2016;5(9):390-99.
[11] Rodrigues C, Joshi P, Jani S, Alphonse M, Radhakrishnan R, Mehta A. Detection of β-lactamases in nosocomial gram-negative clinical isolates. Indian Journal of Medical Microbiology. Elsevier BV; 2004;22(4):247-50.
[12] Nandagopal B, Sankar S, Sagadevan K, Arumugam H, Jesudason M, Aswathaman K, et al. Frequency of extended spectrum β-lactamase producing urinary isolates of gram-negative bacilli among patients seen in a multispecialty hospital in Vellore district, India. Indian Journal of Medical Microbiology. 2015;33(2):282-85.
[13] Bajpai T, Pandey M, Varma M, Bhatambare G. Prevalence of extended spectrum beta-lactamase producing uropathogens and their antibiotic resistance profile in patients visiting a tertiary care hospital in central India: Implications on empiric therapy. Indian Journal of Pathology and Microbiology. 2014;57(3):407.
[14] Grover N, Sahni AK, Bhattacharya S. Therapeutic challenges of ESBLS and AmpC beta-lactamase producers in a tertiary care center. Medical Journal Armed Forces India. 2013;69(1):04-10.
[15] Coudron PE, Moland ES, Thomson KS. Occurrence and detection of AmpC Beta-Lactamases among *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis* isolates at a Veterans Medical Center. Journal of Clinical Microbiology. American Society for Microbiology. 2000;38(5):1791-96.
[16] Gomatheswari SN, Jeyamurugan T. Bacteriological profile and the antibiotic susceptibility pattern of microorganisms isolated from pus/wound swab isolates in patients attending a tertiary care hospital in South India. International Journal of Current Microbiology and Applied Sciences. 2017;6(10):1405-13.
[17] Maheswary D, Chitralekha S. Prevalence and antibiotic susceptibility in *Proteus* species isolated from diverse clinical samples in a tertiary care hospital. Sch J App Med Sci. 2018;6(11):4328-32.
[18] O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus, Providencia,* and *Morganella*. Clinical Microbiology Reviews. American Society for Microbiology. 2000;13(4):534-46.
[19] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 2021.
[20] Maraskolhe DL. Comparision of three Laboratory tests for detection of Ampc ß lactamases in *Klebsiella* species and *E.coli*. J Clin Diagn Res. 2014;8(6):DC05-08.
[21] Amjad A, Mirza Ia, Abbasi S, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iran J Microbiol. 2011;3(4):189-93.
[22] Cury A, Andreazzi D, Maffucci M, Caiaffa-Junior H, Rossi F. The modified hodge test is a useful tool for ruling out *Klebsiella pneumoniae* carbapenemase. Clinics. Fundacao Faculdade de Medicina; 2012;67(12):1427-31.
[23] Bahashwan SA, Shafey HME. Antimicrobial resistance patterns of *Proteus* isolates from clinical specimens. European Scientific Journal. 2013;9(27):188-202.

[24] Prasad RR, Shree V, Sagar S, Kumar S, Kumar P. Prevalence and antimicrobial susceptibility pattern of *Proteus* species in clinical samples. International Journal of Current Microbiology and Applied Sciences. 2016;10;5(4):962-68.
[25] Nirali D, Dipika P. Prevalence and antibiotic susceptibility pattern of *Proteus* species from various clinical samples coming to a tertiary care hospital, Rajkot. MedPulse International Journal of Microbiology. 2020;15(3):14-18.
[26] Pal N, Sharma N, Sharma R, Hooja S, Maheshwari K. 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):243-52.
[27] Feglo PK, Gbedema SY, Quay SNA, Adu-Sarkodie Y, 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.
[28] 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.
[29] 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):293-94.
[30] Al-Bassam WW, Kazaz AKA. The isolation and characterisation of *Proteus* mirabilis from different clinical samples. Journal of Biotechnology Research Center. 2013;7(2):24-30.
[31] De Champs C, Bonnet R, Sirot D, Chanal C, Sirot J. Clinical relevance of *Proteus mirabilis* in hospital patients: A two year survey. J Antimicrob Chemoth. 2000;45:537-39.
[32] 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.
[33] 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.
[34] Kengne M, Dounia AT, Nwobegahay JM. Bacteriological profile and antimicrobial susceptibility patterns of urine culture isolates from patients in Ndjamena, Chad. Pan African Medical Journal. 2017;28.
[35] Preethishree P, Rai R, Kumar KV, Pai KBA, Bhat UP. Uropathogens and their antibiotic susceptibility pattern at a tertiary care teaching hospital in Coastal Karnataka, India. International Journal of Current Microbiology and Applied Sciences. 2016;5(1):23-31.
[36] Alexis A, Sakthivennila M. Bacteriological spectrum and their antimicrobial susceptibility pattern in diabetic ulcer patients attending the tertiary care hospital to facilitate the reduction in morbidity and amputation. International Journal of Current Microbiology and Applied Sciences. 2018;7(05):1465-79.
[37] Kargar M, Jahromi M, Najafi A, Ghorbani-Dalini S, Kargar M. Molecular detection of ESBLs production and antibiotic resistance patterns in gram negative bacilli isolated from urinary tract infections. Indian Journal of Pathology and Microbiology. 2014;57(2):244.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 18, 2021
- Manual Googling: Nov 05, 2021
- iThenticate Software: Nov 20, 2021 (8%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Aug 14, 2021**
Date of Peer Review: **Sep 15, 2021**
Date of Acceptance: **Nov 06, 2021**
Date of Publishing: **Jan 01, 2022**