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Original Research Article

Antimicrobial Susceptibility Pattern and Detection of Extended Spectrum Beta Lactamase Production in Proteus Species Isolated From Various Clinical Specimens at Government Medical College, Kota (Rajasthan)

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Conflict of interest: Nil

Abstract:

Background: Proteus species is commonly associated with hospital as well as community acquired infections. The increasing resistance of Proteus spp to commonly used antibiotics with sensitivity only to reserve drugs such as Imipenem, Ceftazidime-Clavulanic acid, Piperacillin-Tazobactam is one of the most challenging tasks which is faced in clinical practice. The prevalence of ESBL producing Proteus varies from 19.4% to 69.4%.

Aim and Objectives: The aim of this study was to detect extended spectrum beta lactamase production and assess the antimicrobial susceptibility pattern of ESBL producing bacteria in comparison with non-ESBL producers in Proteus species isolated from various clinical specimens at Govt. Medical College & A.G. of Hospitals, Kota.

Material and Methods: A total of 100 non-duplicate Proteus species obtained from various clinical samples like urine, blood, pus, sputum, endotracheal aspirate and body fluids (pleural, ascitic, peritoneal and CSF) etc. were taken for the study from November 2020 to October 2021 and identification was done as per the standard biochemical identification methods. Antimicrobial susceptibility was performed by Kirby–Bauer disc diffusion method. ESBL production was detected by modified double-disc synergy test, confirmatory disk diffusion test and E tests.

Result: Three Proteus species isolated were: Proteus mirabilis 63% (63/100), Proteus vulgaris 34% (34/100), and Proteus penneri 5% (5/100). Proteus species was most commonly isolated from pus (53%) followed by urine (28%), ear swab (7%), blood (5%), vaginal swab (4%) and sputum (3%) respectively. Maximum prevalence was seen in 31-45 years age group (25%) and minimum prevalence in >75 years age group (5%). Males were found to be more vulnerable than females in acquiring Proteus infections. Out of 100 Proteus isolates 69% were isolated from IPD cases and 31% were from OPD cases. Out of 100 Proteus isolates, 63 were positive for ESBL production by screening method, while 61 were positive by DDST method, 63 were positive by CDT method and 61 were positive for ESBL production by E test method. ESBL producers were most susceptible to imipenem (90.44%) followed by piperacillin-tazobactam combination (76.20%). P.penneri was the most resistant species.

Conclusion: This study highlighted the increased prevalence of Proteus mirabilis when compared to Proteus vulgaris and Proteus penneri. Species Proteus penneri was more drug resistant when compared to Proteus mirabilis and Proteus vulgaris. Piperacillin tazobactam and Imipenem were effective in treating the resistant Proteus species to prevent unnecessary use of antimicrobial agents, increasing drug resistance and long duration of hospital stay.

Keywords: Speciation, Antimicrobial susceptibility, ESBL.

Introduction

The genus Proteus belongs to the family Enterobacteriaceae and is placed under the tribe Proteeae which also includes Morganella and Providentia. Members of this genus are pleomorphic, motile, aerobic and facultative anaerobic, Gram negative bacilli and include 5 named species P. mirabilis, P. vulgaris, P. myxofaciens, P. hauseri, P. penneri, and 3 unnamed genomospecies.[1] The organisms of the Proteus group have been known since the earliest days of bacteriology and constitute an important part of the flora of decomposing organic matter of animal

origin.[2] Owing to the varied mode of transmission such as soil, contaminated food, water, equipments, intravenous solutions, the hands of patients and healthcare personnels, these pathogens cause infection in different sites of the body.[3] They are opportunistic pathogens mainly associated with urinary, skin and soft tissue infections. Proteus species are commonly associated with nosocomial infections.[4]

The distinctive feature of many of the Proteus species is swarming on blood agar.[3] Proteus has a distinct odour that is often referred to as a "chocolate cake" or "burnt chocolate" smell. However, for safety reasons, smelling plates is strongly discouraged in the clinical laboratory.[5]

Proteus infections rank as third cause of health care associated infections and the reported prevalence worldwide is between 9.8% and 14.6%.[5] P.mirabilis causes 90% of all Proteus infections which is both community and hospital acquired, whereas P.vulgaris and P.penneri are isolated largely from various hospitalised individuals who are having an underlying disease. [6] About 5% of cases of hospital-acquired UTIs are due to Proteus spp and 10–15% cases of complicated UTI are mainly those that are associated with catheterization are due to Proteus spp..[7] Proteus species also accounts for around 10% of wound infections and causes significant morbidity and mortality. [8] The other isolates like P.myxofacien. And P.hauseri is very rarely isolated. Investigations on characterization or pathogenicity of P.myxofaciens have not been reported. Caroline O' Hara has reported 1 case of isolation of Proteus hauseri.[9]

The sensitivity pattern of Proteus to Penicillin and Cephalosporins is determined in part by mechanisms of intrinsic resistance and partly by extrinsic resistance such as by producing β lactamases as these antibiotics are the most frequently used antibiotics for empirical treatment.[10] The prevalence of ESBL producing Proteus vary from 19.4% to 69.4%.[11,12]. According to the Bush Jacoby and Medeiros functional classification, ESBL is placed under the group 2be, and under class A, of Ambler's molecular classification.[13]These are plasmid borne, evolved from point mutations that vary the configuration of the active site of β-lactamases TEM-1,TEM-2 and SHV-1.[14]

Indiscriminate intake of antibiotics in the health care settings provides selective pressure leading to higher prevalence of resistant bacteria which is very common in developing countries like India. These species are potential reservoirs of resistant genes that might possibly be transferred to other bacterial pathogens.[15]

Material and Method

Specimen Collection

A total of 100 non-duplicate Proteus species obtained from various clinical samples like urine, blood, pus, sputum, endotracheal aspirate and body fluids (pleural, ascitic, peritoneal and CSF) etc. of patients attending out-patient department or inpatient department of all the associated group of hospitals (MBS Hospital, NHMC and JK Lon Hospital) were taken for the study and identification was done as per the standard biochemical identification methods.

Cultivation and Identification

All specimens were processed according to standard operating procedure of laboratory. Organisms were grown on nutrient agar, blood agar and mac conkey agar and were identified by their Motility testing by hanging drop method**,** Colony characteristics on solid media, Gram's staining of the isolated colonies and Battery of biochemical reactions including: Catalase test, oxidase test, indole production, methyl red, Voges-Proskauer test, citrate utilization test, urease test, triple sugar iron (TSI) test, phenylalanine deaminase test, decarboxylation of amino acids, sugar fermentation test. [16,17,18,19]

Antimicrobial Susceptibility Test

All the identified proteus isolates were then subjected to antibiotic susceptibility testing by Kirby Bauer disk diffusion method according to CLSI guidelines using Mueller-Hinton agar medium and commercial antibiotic discs (HIMEDIA).[20] The antimicrobial agents used were: Ampicillin(10), Amoxicillin clavulanate(20/10), Ceftriaxone (30), Ceftazidime (30), Aztreonam(30), Cefepime (30), Gentamicin (10), Amikacin (30), Imipenem (10), Ciprofloxacin (5), Piperacillin (100), Piperacillin-Tazobactum (100/10). The inocula were prepared by growing the various Proteus species on separate agar plates and colonies from the plate were transferred with inoculating loop into 3 ml of normal saline in a test tube.

The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar (Oxoid Cambridge, UK) plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid.

The wet swab was then used to inoculate the Muller-Hinton agar by evenly streaking across the surface. By means of Disc Dispenser (Oxoid Cambridge, UK), the antibiotic discs were applied to the surface of the inoculated agar and the plates were incubated overnight at 37ºC. The diameter of zone of growth-inhibition observed was measured and compared to the chart provided by National

Committee for Clinical Laboratory Standards (NCCLS).

Detection of ESBL production by phenotypic methods:

Screening test for ESBL: As recommended by the CLSI the study isolates were tested for their susceptibility to the third generation Cephalosporins (3GCs) by using disk diffusion method. If the zone diameter of < 22 mm for Ceftazidime, < 27 mm for Cefotaxime and< 22 mm for Cefpodoxime were noted, the strain was suspected for ESBL production and Only screen positive isolates were selected and Confirmatory test ESBL production was done.

Confirmatory test for ESBL:

The Double disk synergy test: As per the British society for antimicrobial chemotherapy guidelines DDST test was done. A plate of Mueller Hinton Agar was inoculated with the test strain. Disks of Ceftazidime, Cefotaxime and Amoxyclav (30 µg Amoxycillin and 10 µg Clavulanic acid) were kept at a distance of 20 mm from center to center in a straight line, with the Amoxyclav disk in the middle. The plates were incubated aerobically at 37ºC for 12-18 hours. If the enhancement of the zone of inhibition is greater than 5 mm on the Amoxyclav side of the disc when compared to that which was seen on the side without Amoxyclav then the isolates were confirmed as ESBL producers.

Confirmatory disk diffusion test (CDT): A plate of MHA was inoculated with the test strain. The (30 µg) Cefotaxime disk alone and in combination with Clavulanic acid (Cefotaxime + Clavulanic acid, 30/10 µg discs) was applied onto the plate. An increase of ≥ 5mm in the zone of inhibition of the combination discs in comparison to the Cefotaxime disk alone was considered to be a marker for ESBL production. Similarly the test was done with Ceftazidime and in combination with Clavulanic acid (Ceftazidime +Clavulanic acid 30/10 µg).

Epsilometer Test: The isolated Proteus species was streaked onto the Mueller Hinton agar plate and after few minutes of drying an E-strip containing a concentration gradient of Ceftazidime (0.5µg - 32µg/ml) and Ceftazidime plus clavulanic acid (0.064-4µg/ml) was placed on it and incubated at 37°C for 18-24 hours. After 24 hours of the incubation period MIC was noted. An MIC value of Ceftazidime/Ceftazidime-clavulanate ratio ≥ 8 was taken as ESBL positive. The following strains were used as quality control strains as per CLSI guidelines:

Positive control strain - Klebsiella pneumoniae ATCC 700603

Negative control strain - E . coli ATCC 25922.

Results and Discussion

During the study period of 1 year from November 2020 to October 2021, a total of 100 Proteus species were isolated from various clinical samples of patients from OPD and IPD of all the associated group of hospitals (MBS Hospital, NHMC and JK Lon Hospital). Proteus species was most commonly isolated from pus (44%) followed by urine (28%), ear swab (07%), blood (05%), vaginal swab (4%) and sputum (3%) respectively.

Table 1: Specimen wise distribution of the Proteus species

S. No	Specimen	P. mirabilis	P. vulgaris	P. penneri	Total	Percentage
1.	Pus	28	21	4	53	53%
2.	Urine	19	8	1	28	28%
3.	Ear Swab	5	2	-	7	7%
4.	Blood	4	1	-	5	5%
5.	Vaginal Swab	3	1	-	4	4%
6.	Sputum	2	1	-	3	3%
7.	**Total**	**61**	**34**	**05**	**100**	**100%**

The most common Proteus species isolated was Proteus mirabilis (61%) followed by Proteus vulgaris(24%) and Proteus penneri (5%).

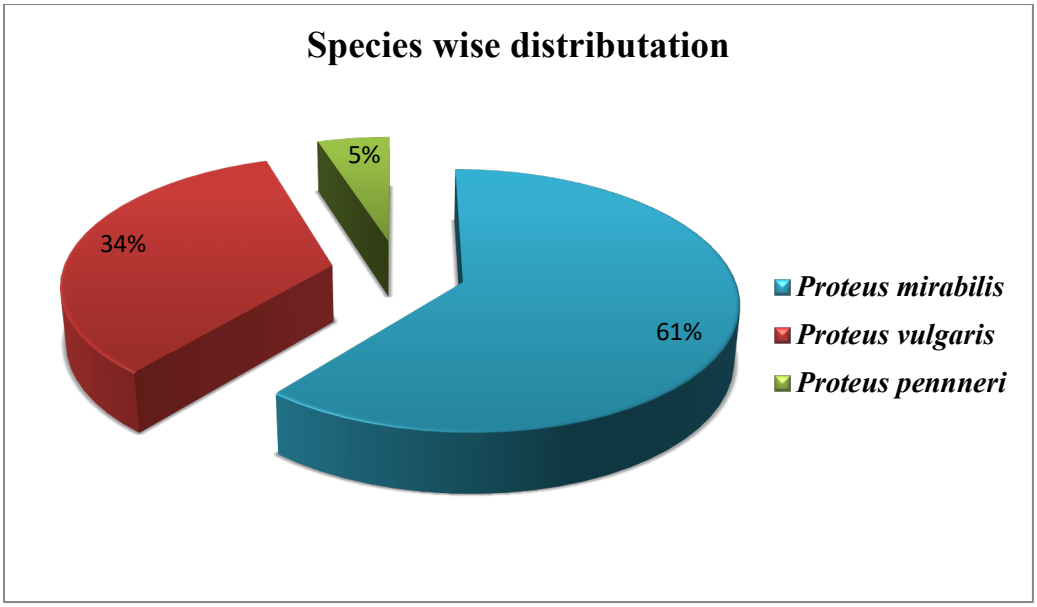

Figure 1: Species wise distributation

Out of 100 Proteus isolates, 25 were between the age group of 31 to 45 years, 24 were between the age group of 16 to 30 years and around 22 between the age group of 46 to 75.Maximum prevalence was seen in 31-45 years age group (25%) and minimum prevalence in >75 years age group (5%). Mean age of study group was 43.96 years.

Maximum prevalence of P.mirabilis was in 31-45 years age group (16%) and minimum prevalence in >76 years age group (4%).Maximum prevalence of P.vulgaris was in 16-30 years age group (11%) and minimum prevalence in 0-15 years &>76 years age group (1 %).Maximum prevalence of P.penneriwas in 31-45 years age groups (2%) and no P.penneri isolate was found in 0-15 years &>76years age group.

Out of 100 Proteus isolates, 59 were males and 41 were females.

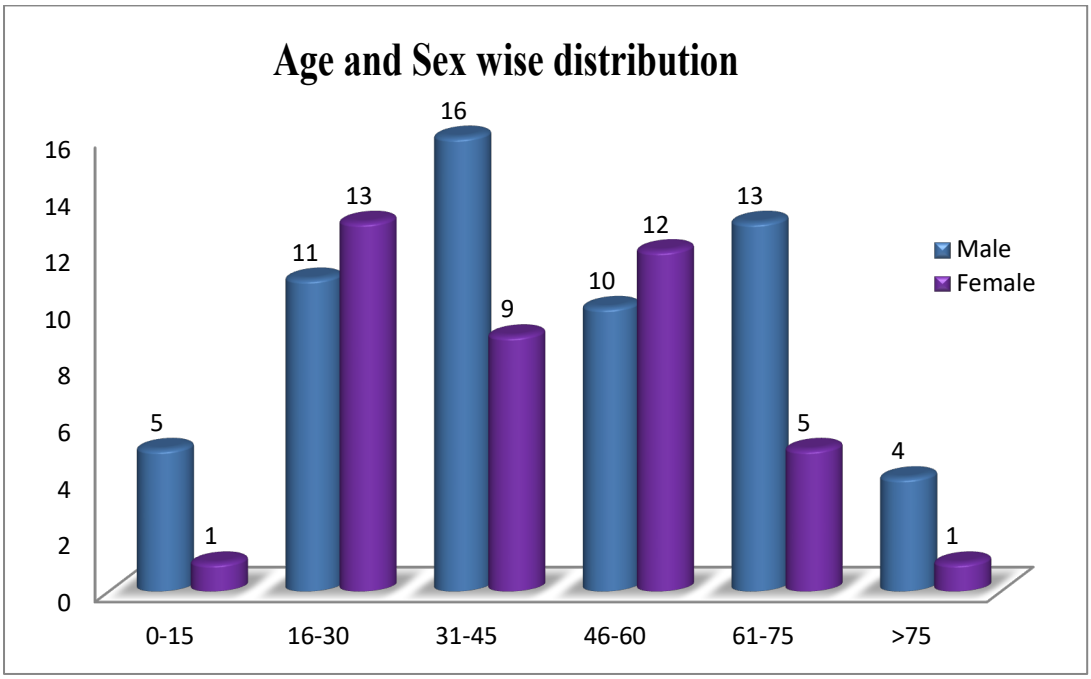

Figure 2: Age and sex wise distribution

Out of 100 Proteus isolates 69% were isolated from IPD cases and 31% were from OPD cases. 61% P.mirabilis were isolated from inpatient and 21% were from outpatient.24% P.vulgaris were isolated from inpatient and 10% were from outpatient.5% P.penneri were isolated from inpatient and none were from outpatient.

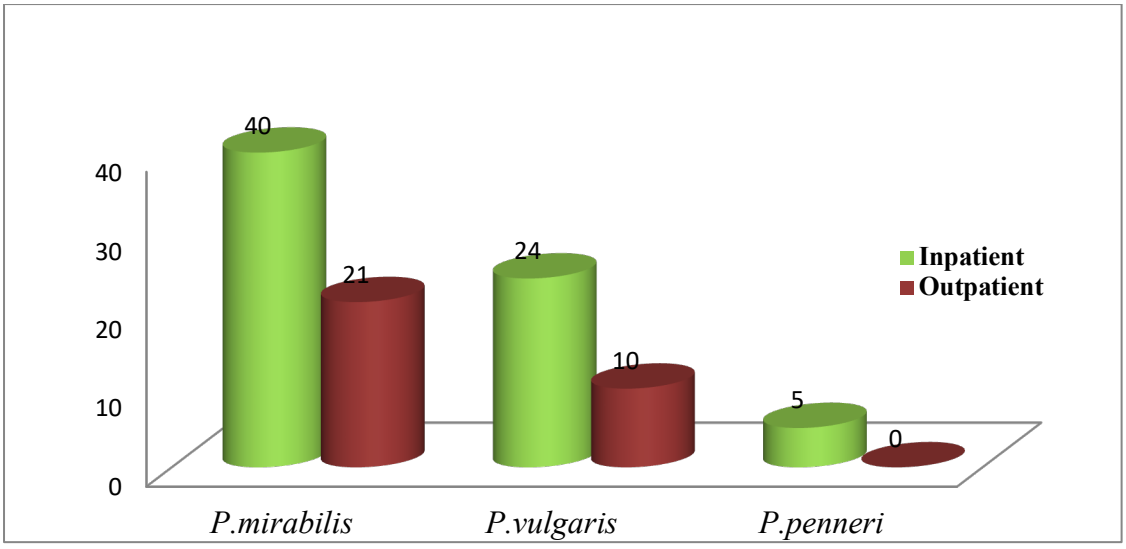

Figure 3:

The rate of isolation of the Proteus species was highest from the Urology ward (19%) followed by Burn ward (15%), Surgery ward (12%), Ortho (10%), ENT and medicine (4%), O&G(3%) and Pediatrics (2%).

Table 2: Ward& OPD wise distribution of Proteus isolates

S.No	Ward	Total	Percentage
1.	Burn	15	15%
2.	Surgery	12	12%
3.	Ortho	10	10%
4.	Urology	19	19%
5.	ENT	04	04%
6.	Medicine	04	04%
7.	O&G	03	03%
8.	Pediatrics	02	02%
9.	OPD	31	31%
10.	**Total**	**100**	**100%**

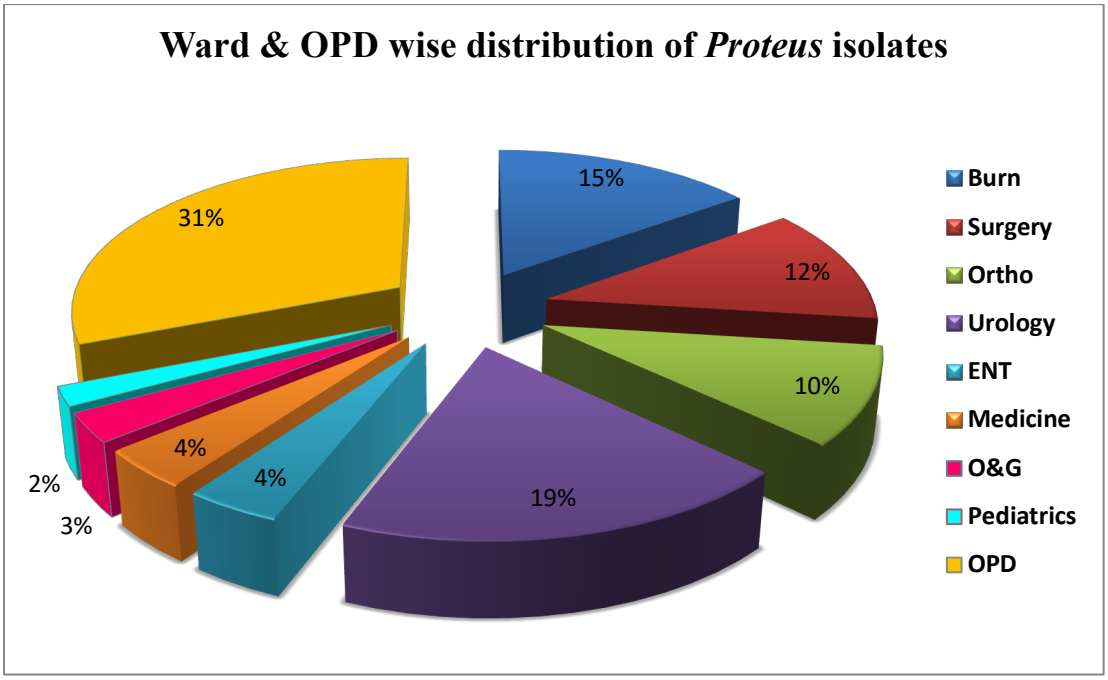

Figure 4: Ward & OPD wise distribution of Proteus isolates

The Proteus isolates were most susceptible to Imipenem (94%) and Piperacillin Tazobactum (92%).

Table 3: Antibiotic susceptibility pattern of Proteus isolates

S. No.	Antibiotics	Number(n=100)	Percentage (%)
1.	Ampicillin(10)	39	39
2.	Amoxyclav(20/10)	52	52
3.	Ceftriaxone (30)	32	32
4.	Ceftazidime (30)	32	32
5.	Aztreonam (30)	38	38
6.	Cefepime (30)	44	44
7.	Gentamicin (10)	40	40
8.	Amikacin (30)	33	33
9.	Imipenem (10)	94	94
10.	Ciprofloxacin (5)	36	36
11.	Piperacillin (100)	27	27
12.	Piperacillin-Tazobactum (100/10)	92	92

Table 4: Antibiotic susceptibility pattern of ESBL producer in comparison to ESBL non producer

S. No.	Antibiotics	Sensitivity of ESBL producer (%)	Sensitivity of ESBL non producer (%)
1.	Ampicillin(10)	19.10	48.70
2.	Amoxyclav(20/10)	46.04	70.27
3.	Ceftriaxone (30)	12.70	67.57
4.	Ceftazidime (30)	17.50	72.98
5.	Aztreonam (30)	19.05	70.04
6.	Cefepime (30)	36.51	86.50
7.	Gentamicin (10)	31.75	64.87
8.	Amikacin (30)	49.26	81.10
9.	Imipenem (10)	19.44	94.60
10.	Ciprofloxacin (5)	32.84	62.20
11.	Piperacillin (100)	15.90	51.40
12.	Piperacillin-Tazobactum (100/10)	76.20	83.80

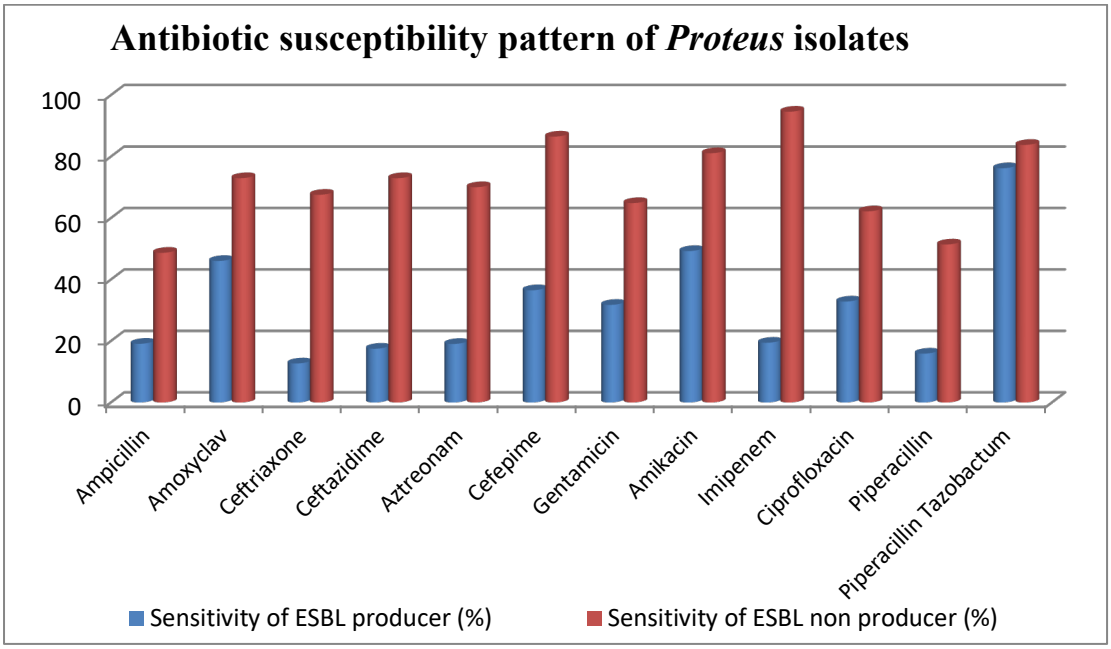

Figure 5: Antibiotic susceptibility pattern of Proteus isolates

Detection of ESBL:

By Phenotypic Methods:

Screening Test: According to the screening test by Disk Diffusion method the percentage of resistance was 63%.

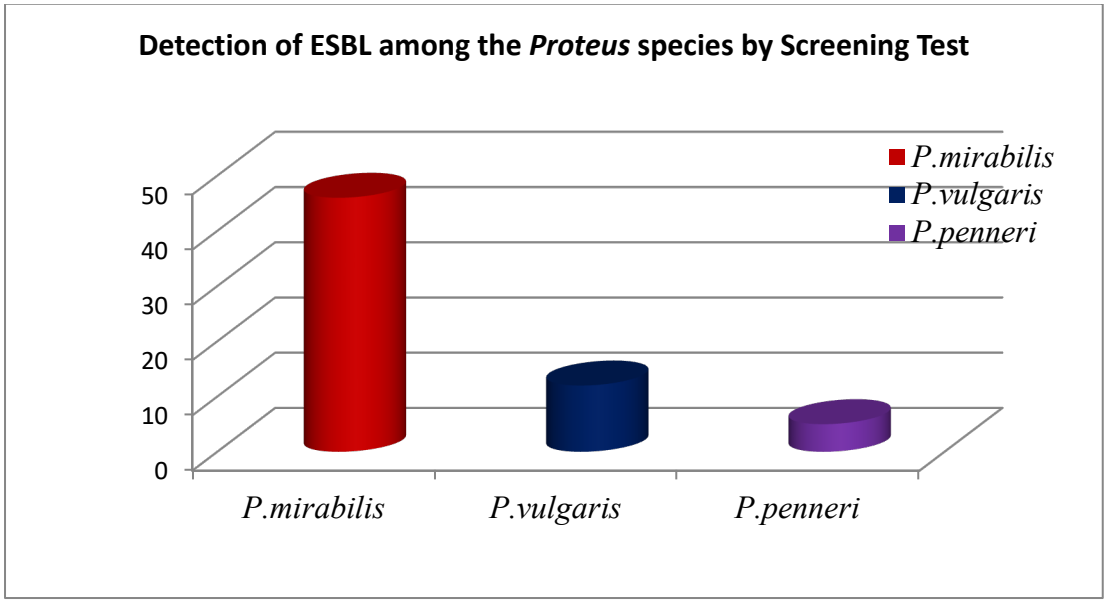

Figure 6: Detection of ESBL among the Proteus species by Screening Test

Confirmatory Methods:

- By CDT method, out of 100 Proteus isolated species, Proteus mirabilis was the most resistant species with about 46% of ESBL production, Proteus vulgaris showed 12% ESBL production and P.penneri showed 5% ESBL production.
- By DDST method, out of 100 Proteus isolated species, Proteus mirabilis was the most resistant species with about 44% of ESBL production, Proteus vulgaris showed 12% ESBL production and P.penneri showed 5% ESBL production.
- By E TEST method, out of 100 Proteus isolated species, Proteus mirabilis was the most resistant species with about 44% of ESBL production, Proteus vulgaris showed 12% ESBL production and P.penneri showed 5% ESBL production.

Comparison of ESBL Detection by Phenotypic Methods:

Table 5: ESBL Detection by Phenotypic methods

Screening Test	CDT Test	DDST	E-Test
63	63	61	61

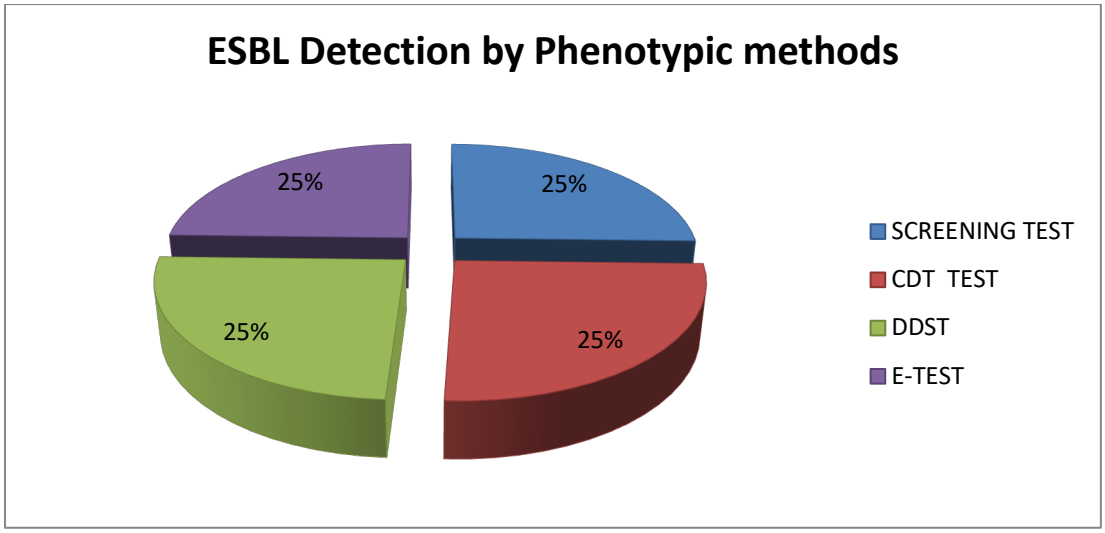

Figure 7: ESBL Detection by Phenotypic methods

Conclusion:

In conclusion, this study highlighted the increased prevalence of Proteus mirabilis when compared to Proteus vulgaris and Proteus penneri. The species Proteus penneri was more drug resistant when compared to Proteus mirabilis and Proteus vulgaris. For ESBL detection Combined Disk Test and E-Test were found to be very effective. Combined Disk test is a simple, easy test to perform for the confirmation of ESBL production. E-Test is more accurate in detecting ESBL prevalence. Piperacillin tazobactam and Imipenem were effective in treating the resistant Proteus species. So this study was helpful in treating ESBL producer Proteus species in the community or hospitalized patients. To prevent the spread of ESBL producing strains of Proteus species effective infection control measures like hand hygiene, cohorting of the patients, dedicated patient's equipment, and training of the health care personnel should be followed.

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.[21]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.

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