ISSN: 2395 - 0471

International Journal of Clinical and Biomedical Research
Original Article

SPECIATION AND ANTIBIOGRAM PATTERN OF TRIBE PROTEEAE WITH SPECIAL REFERENCE TO EXTENDED SPECRUM BETA LACTAMASE (ESBL) AND CARBAPENEMASE DETECTION

SH SHIFA MEHARAJ[1], *PRIYADARSHINI SHANMUGAM[2], SHAMEEM BANU AS[3].

[1]PG Student, [2]Professor, [3]Professor & Head Department of Microbiology, Chettinad Hospital and Research Institute, Rajiv Gandhi Salai, Padur, Kanchipuram Dist- 603103.

*Corresponding author email: priyadarshini0018@gmail.com

Received: 12th Mar 2015, Accepted: 23rd Mar 2015.

ABSTRACT

Background: *Proteus* is a common uropathogen causing urinary tract infections in catheterised patients and those with urinary tract abnormalities. It may also lead to pyelonephritis, renal stones & bacteraemia. Multidrug resistant *Proteeae* isolates are major problem in treating nosocomial infections. Beta lactamase production is being increasingly demonstrated in most of the *Proteus* species. Apart from ESBL, Carbapenemase production is also emerging, thereby limiting the treatment options. **Aim**: To isolate, speciate and study the antibiotic resistance pattern of the *Proteeae* isolates. To identify the extended spectrum beta lactamase and carbapenemase producing strains of *Proteeae* isolates by employing phenotypic methods. **Materials and Methods**: A total of 145 isolates of *Proteus* species isolated from different clinical samples- urine, pus and sputum, were included in this study. Antibiotic sensitivity testing was performed by Kirby-Bauer disk diffusion method. Screening tests for ESBL & Carbapenemase production was confirmed by Disk diffusion method and Modified Hodge test respectively. **Results**: Out of the 145 *Proteeae* isolates, from various clinical samples 70 were from wound swabs, 62 from urine, and 13 from respiratory specimens. The species were identified as 64 *P.mirabilis*, 48 *P.vulgaris*, 19 *M.morganii*, 5 *Prov.stuartii* and 9 *Prov.rettgeri.* Different antibiotic resistance patterns were observed in different species. *Providencia* species showed resistance to most of the antibiotics than the *Proteus* species and *M morganii.* Of the total, 52 (36%) were ESBL producers. Among the ESBL producers 6 (11.5%) were Carbapenamase producers. **Conclusion**: The increasing incidence of multi drug resistant strains in Tribe *Proteeae* has made antimicrobial susceptibility testing more important. Avoidance of indiscriminate use of antibiotics is the first step in prevention of newly emerging drug resistant strains.

KEYWORDS: *Proteeae,* ESBL, Carbapenemase, Double disk Synergy test, Modified Hodge test.

INTRODUCTION

The Tribe *Proteeae* consists of Gram negative bacilli belonging to the family Enterobacteriaceae and it has three genera: *Proteus, Morganella* and *Providencia*. They are usually found in the environment causing hospital acquired and community acquired infections. Stamm in his study said that Proteus species is the third most common pathogen causing nosocomial infection (12%)[1]. They are mostly associated with urinary tract infections following catheterization especially in the patients who have structural or functional abnormalities. 90 % of *Proteus* infections are due to Proteus mirabilis. This is the most common species causing pyelonephritis, urolithiasis (bladder and kidney stone) leading to complications [2]. In the individuals with the long term indwelling catheters, *Proteus mirabilis* is the second leading cause of Catheter associated urinary tract infection (15%). This was studied by Jacobsen et a[3].

Due to the emerging drug resistance, treatment of *Proteeae* infections is difficult. Multidrug resistant *Proteeae* isolates are major problem in treating nosocomial infections. Luzzaro et al., 2009; D'Andrea et al., 2011 have reported the spread of the

multidrug resistant *P.mirabilis* in Europe[4,5]. Extended spectrum Beta lactamase (ESBL) production is being increasingly demonstrated in most of the *Proteeae* species. This is manifested as resistance to third generation cephalosporins (ceftazidime, cefotaxime and ceftriaxone) and monobactams but not cephamycins and carbapenems. 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 [6]. ESBL producing organisms lead to treatment failure and spread of the resistance among the different strains. Therefore, screening for ESBL is necessary in order to provide effective treatment.

Apart from ESBLs, carbapenemase production is also emerging among the multidrug resistant organisms. Carbapenem resistance is due to the altered penicillin binding proteins or outer membrane proteins. These are also Beta lactamases which hydrolyses not only the carbapenem antibiotics but also the penicillins, cephalosporins, and the monobactams. Hence detection of the carbapenemase production is also mandatory before prescribing antimicrobials. As per Ambler's classification, they are classified as class A, B and D[6].

Earlier, ESBL production and Carbapenemase were rarely observed in *Proteeae* isolates but now the frequency of ESBL detection among the *Proteus* and *Providencia* species has increased. Therefore, this study demonstrates the susceptibility pattern of different species of Tribe *Proteeae* to commonly prescribed antibiotics and the prevalence of ESBL and Carbapenemase enzyme in the *Proteeae* isolates by phenotypic confirmatory methods.

MATERIALS AND METHODS

A total of 145 isolates of *Proteus* species from different clinical samples like urine, pus, sputum, endotracheal aspirates, collected from patients suffering from infections, over a period of 1 year from January 2013 to January 2014 were included in this study. The study was carried out in the Department of Microbiology, Chettinad Hospital and Research Institute, Kelambakkam, Chennai. Consent was obtained from the patients. The Institutional Ethical Committee approval was obtained prior to commencing the study. Species level identification of the members of the genus *Proteus, Morganella and Providencia was* made based on the property of swarming and biochemical tests like Catalase, Oxidase, Indole, Methyl red, Voges Proskauer, Citrate, Urease, Triple sugar iron agar, H_2S production, Phenylpyruvic acid test, ornithine decarboxylation, and sugar fermentation test. Antibiotic susceptibility testing for all the three genera was determined by Kirby-Bauer Disk Diffusion method, and diameter of the zone of inhibition was measured and interpreted according to the CLSI guidelines[7]. The isolates that were resistant to third generation cephalosporins in were tested for the production of Extended Spectrum Beta Lactamase by double disc synergy test.

Detection of ESBL production:

The double disk synergy test was performed by drawing a lawn culture of the test strain with 0.5 McFarland standard on the Muller Hinton agar plate. Then a third generation cephalosporin disk and a combination disk containing cephalosporin plus clavulanate were placed at a distance of 15mm from each other (center to center) on the surface of the media with sterile forceps. The disks used were Ceftazidime- 30µg, Ceftazidime-Clavulanic acid- 30µg/10µg and Cefotaxime-30 µg, Piperacillin-tazobactam (100µg/10µg). Then the plates were incubated at 37 °C for 16-18hrs and the diameter of zones of inhibition around the disks was measured. A ≥ 5mm diameter increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid with the zone diameter of the agent when tested alone confirms ESBL production by the strain. Detection of Carbapenemase production.

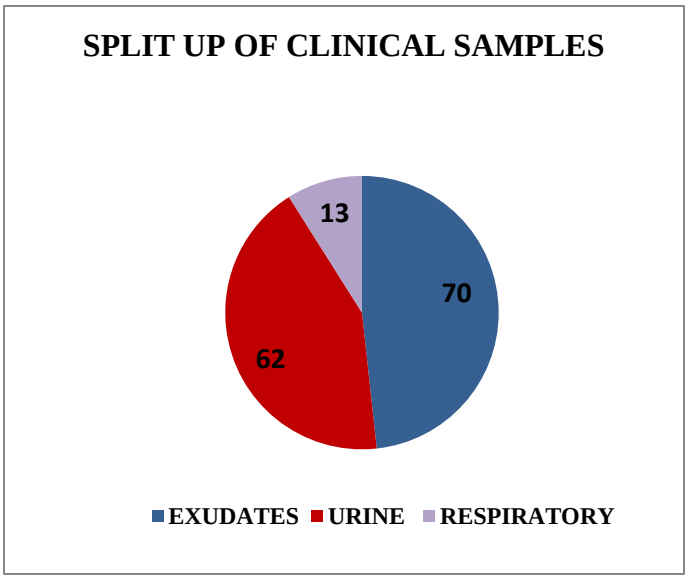
All the ESBL isolates that showed resistance to carbapenems (Imipenem and Meropenem) by disk diffusion and MIC determination method were confirmed for carbapenemase production by using the Modified Hodge Test, the phenotypic test for carbapenemase production. A lawn culture of carbapenem susceptible strain (*E.coli* ATCC 25922) standardised with 0.5 McFarland was swabbed on the Muller Hinton agar plate. A Ertapenem 10 µg disc was placed in the middle of the plate. Suspected carbapenemase producing isolates were streaked from the edge of disk to edge of the plate. Four isolates were tested per plate. The plate was then incubated at 37^0 C for 16-18 hrs. Clover leaf shaped indentation at the

intersection of the isolate and the *E.coli* within the zone of inhibition around the carbapenem disk is considered as positive for carbapenemase production.

RESULTS

Of the 145 *Proteeae* isolates 70 were from pus or wound swabs, 62 from urine, and 13 from respiratory specimens. Among the 70 exudate isolates, 39 (56%) were *P.mirabilis*, 18 (26%) *P.vulgaris*, 4 (6%) *Morganella*, 4 (6%) *Prov.stuartii* and 5 (7%) *Prov.rettgeri.* Among the 62 urine isolates, the most common was *P.vulgaris* 27 (43.5%), followed by 16 (26%) *P.mirabilis*, 14 (22.5%) *Morganella*, 1 (2%) *Prov.stsuartii* 4 (6%) and *Prov.rettgeri*. Among the 13 respiratory isolates, mostly *P.mirabilis* and *P.vulgaris* were isolated - 9 (69%) and 3 (23%) respectively. Only one isolate of *Morganella* morganii was obtained.

Figure 1. Split up of clinical samples in Tribe *Proteeae*



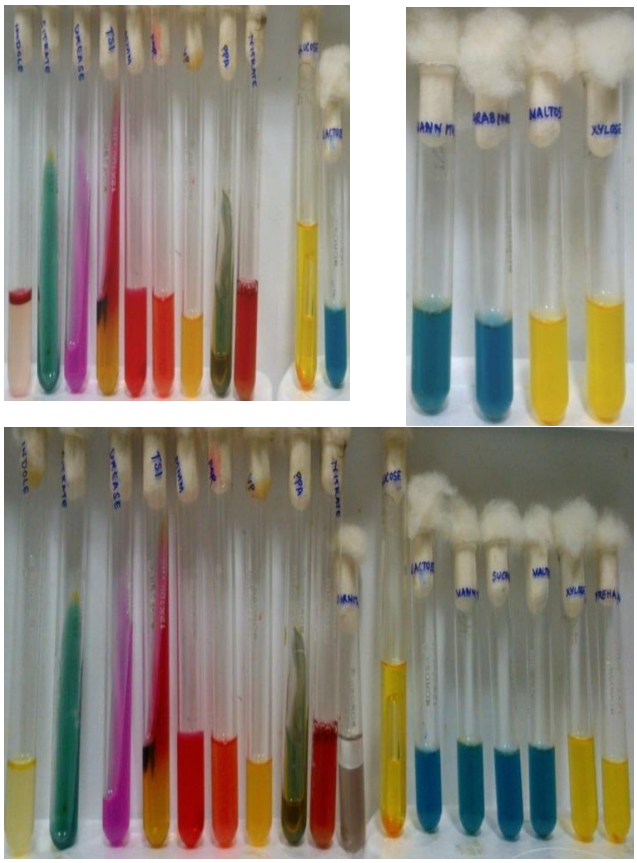


Figure 2. Speciation of *Proteeae* isolates by biochemical tests

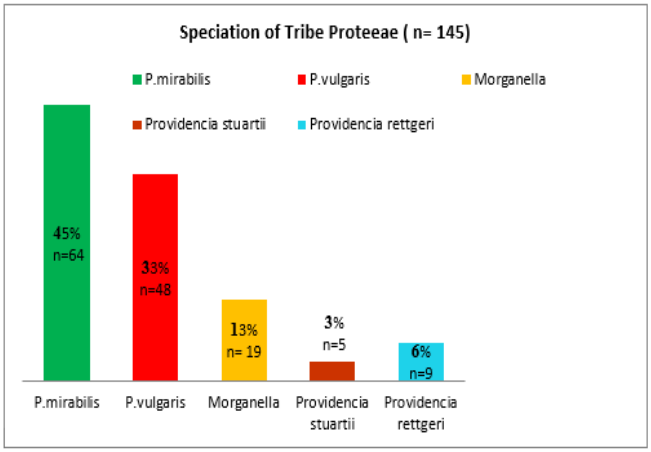


Figure 3a. Speciation of Tribe *Proteeae*

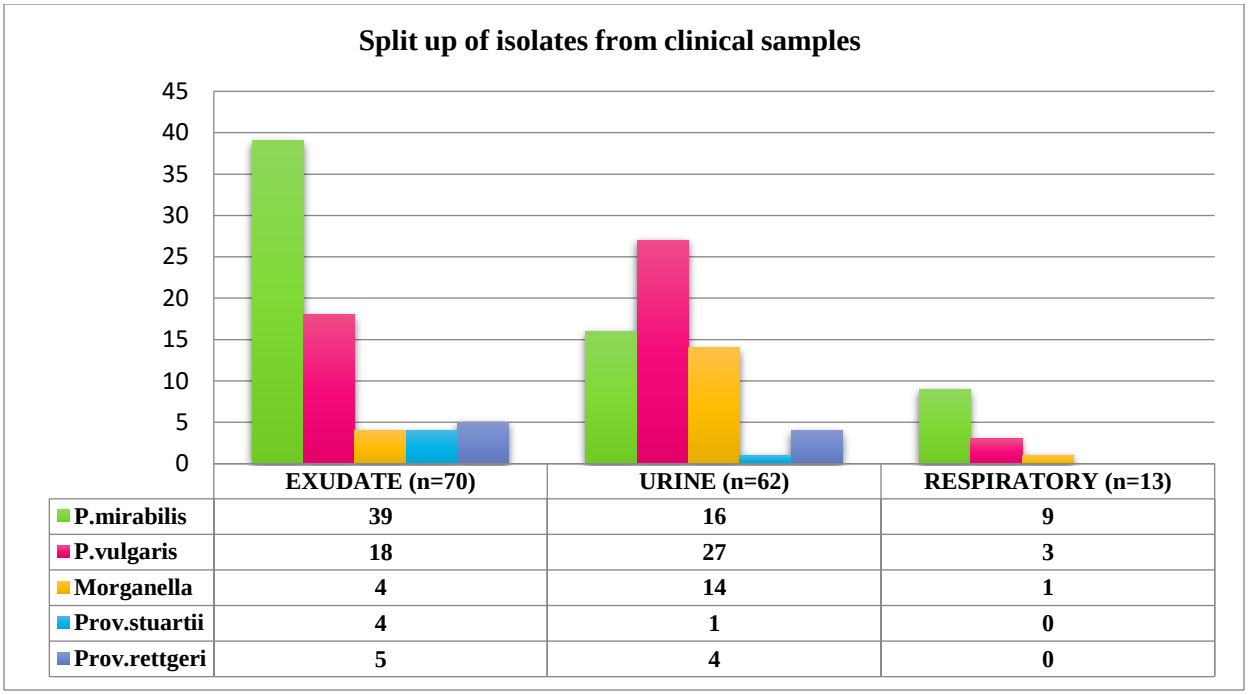


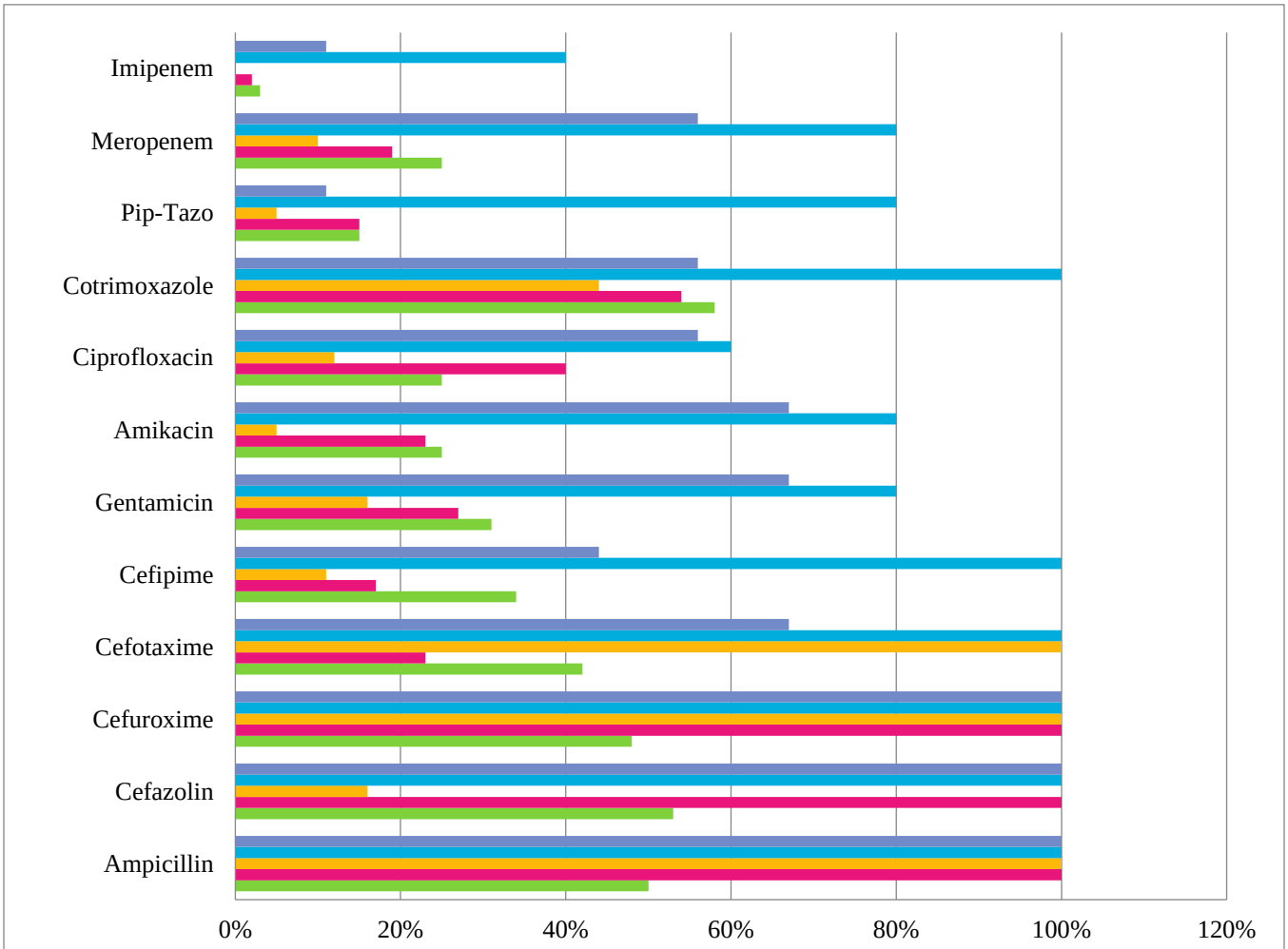
Figure 3b. Speciation of isolates from clinical samples

Table No.1: Antibiotic sensitivity pattern of Tribe *Proteeae*

Antibiotic	Percentage Sensitivity (%) *P.mirabilis* isolates (n=64)	Percentage Sensitivity (%) of *P.vulgaris* isolates (n=48)	Percentage Sensitivity (%) of *Morganella* isolates (n=19)	Percentage Sensitivity (%) of *Prov.rettgeri* isolates (n=9)	Percentage Sensitivity (%) of *Prov.stuartii* isolates (n=5)
Ampicillin	32 (50%)	Intrinsic resistance	Intrinsic resistance	Intrinsic resistance	Intrinsic resistance
Cefazolin	30 (47%)	Intrinsic resistance	Intrinsic resistance	Intrinsic resistance	Intrinsic resistance
Cefuroxime	33 (52%)	Intrinsic resistance	Intrinsic resistance	Intrinsic resistance	Intrinsic resistance
Cefotaxime	37 (58%)	37 (77%)	16 (84%)	3 (33%)	0
Cefipime	42 (66%)	40 (83%)	16 (84%)	4 (44%)	0
Gentamicin	44 (69%)	37 (77%)	16 (84%)	3 (33%)	1 (20%)
Amikacin	48 (75%)	37 (77%)	18 (95%)	3 (33%)	1 (20%)
Ciprofloxacin	48 (75%)	29 (60%)	17 (89%)	4 (44%)	2 (40%)
Cotrimoxazole	27 (42%)	22 (46%)	9 (47%)	4 (44%)	0
Piperacillin-Tazobactam	54 (84%)	22 (46%)	18 (95%)	8 (89%)	1 (20%)
Meropenem	48 (75%)	22 (46%)	17 (89%)	4 (44%)	1 (20%)
Imipenem	63 (98%)	46 (96%)	19 (100%)	8 (89%)	3 (60%)

Figure 3b shows speciation of the *Proteeae* isolates from the clinical samples- exudate 70, urine 62 and respiratory 14. Among the 70 exudate isolates, 39 (56%) were *P.mirabilis*, 18 (26%) *P.vulgaris*, 4 (6%) *Morganella*, 4(6%) *Prov.stuartii* and 5 (7%) *Prov.rettgeri*. Among the 62 urine isolates, the most common was *P.vulgaris* 27 (43.5%), followed by 16 (26%) *P.mirabilis*, 14 (22.5%) *Morganella*, 1 (2%) *Prov.stsuartii*, 4 (6%) *Prov. rettgeri*.

Among the 13 respiratory isolates, *Providencia* species was not isolated. The number of *P.mirabilis* and *P.vulgaris* isolates were 9 (69%) and 3 (23%) respectively. Only one isolate of *Morganella* morganii was obtained.



	Ampicillin	Cefazolin	Cefuroxime	Cefotaxime	Cefipime	Gentamicin	Amikacin	Ciprofloxacin	Cotrimoxazole	Pip-Tazo	Meropenem	Imipenem
P.rettgeri (n=9)	100%	100%	100%	67%	44%	67%	67%	56%	56%	11%	56%	11%
P.stuartii (n=5)	100%	100%	100%	100%	100%	80%	80%	60%	100%	80%	80%	40%
Morganella (n=19)	100%	16%	100%	100%	11%	16%	5%	12%	44%	5%	10%	0%
P.vulgaris(n=48)	100%	100%	100%	23%	17%	27%	23%	40%	54%	15%	19%	2%
P.mirabilis (n=64)	50%	53%	48%	42%	34%	31%	25%	25%	58%	15%	25%	3%

Figure 4. Percentage (%) of resistance of Tribe *Proteeae* isolates to different antibiotics

ESBL producers: The isolates which were resistant to third generation cephalosporins were tested for the production of Extended Spectrum Beta Lactamase (ESBL) by double disk synergy test and the interpretation was recorded as mentioned previously. Among the 145 Tribe *Proteeae* isolates 52 (36%) were ESBL producers. 27 out of 64 *P.mirabilis,* 14 out of 48 *P.vulgaris,* 2 out of 19 *Morganella*, 9 out of *Providencia* species.

Figure 5. ESBL producer in Double disk synergy test

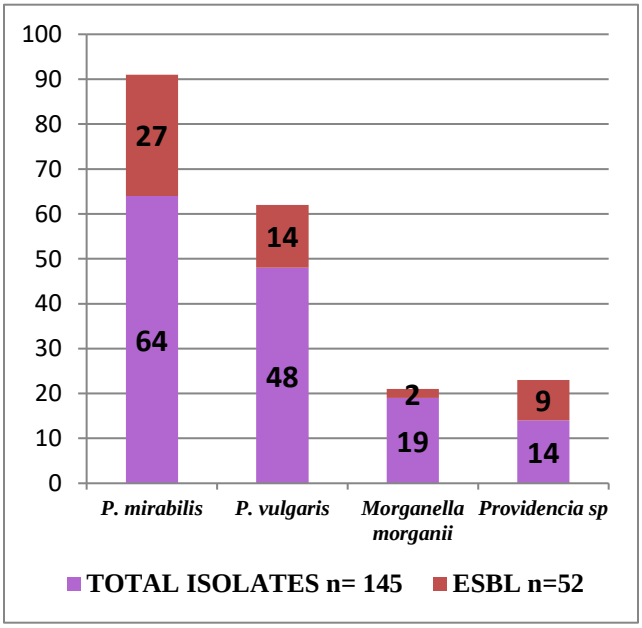


Figure 6. ESBL among different species

Carbapenemase producers among the ESBL strains: Among the 52 ESBL producers, only 6 (11.5%) were found to be resistant to carbapenems (imipenem and meropenem) by disk diffusion method. Carbapenemase production was confirmed by Modified Hodge test as mentioned previously. None of the *Morganella* isolates were found to be resistant. 1 *P.mirabilis*, 1 *Prov.rettgeri* and 2 *P.vulgaris* and 2 *Prov.stuartii.*

Table 2. Carbapenemase production in isolates from different samples

	Urine	Exudate
P.mirabilis	-	1
P.vulgaris	2	-
Prov.stuartii	-	2
Prov.rettgeri	1	-

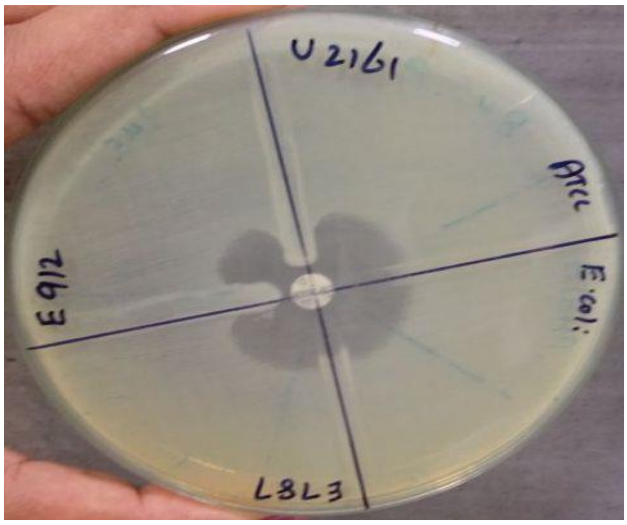
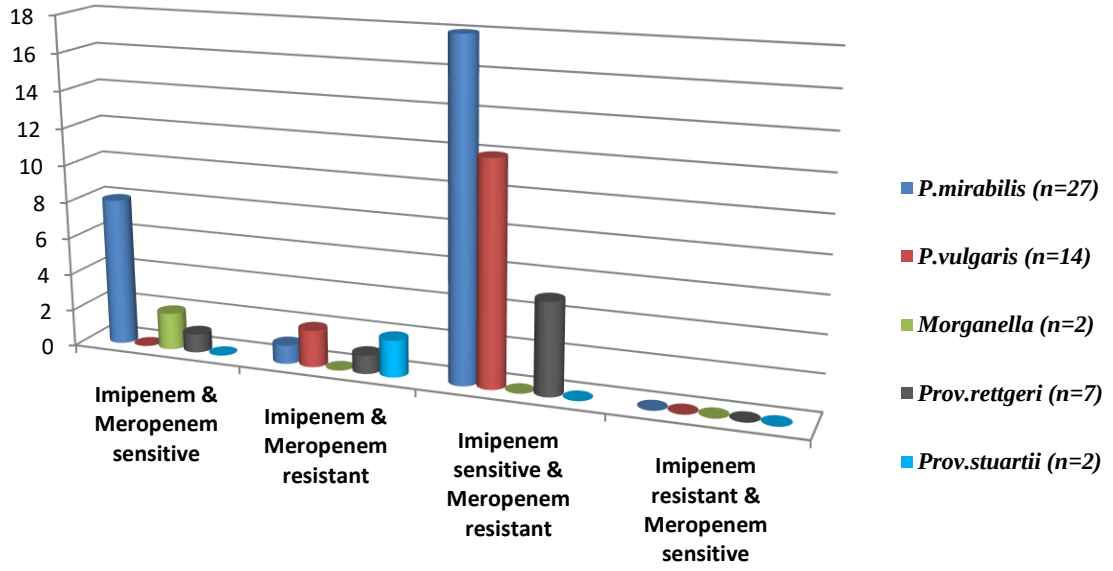


Figure 7. Modified Hodge test for Carbapenemase detection

Clover leaf shaped indentation at the intersection of test organism and the *E.coli* with in the zone of inhibition around the carbapenem disk is considered as positive for carbapenemase production

The isolates which produced ESBL also showed resistance to cotrimoxazole, probably the drug resistance emerging. Among the 52 ESBL isolates, all the ESBL produced *Proteus, Morganella, Providencia* species were resistant to cotrimoxazole.



	Imipenem & Meropenem sensitive	Imipenem & Meropenem resistant	Imipenem sensitive & Meropenem resistant	Imipenem resistant & Meropenem sensitive
P.mirabilis (n=27)	8	1	18	0
P.vulgaris (n=14)	0	2	12	0
Morganella (n=2)	2	0	0	0
Prov.rettgeri (n=7)	1	1	5	0
Prov.stuartii (n=2)	0	2	0	0

Figure 8. Resistance pattern to carbapenems among the ESBL producing *Proteeae* isolates

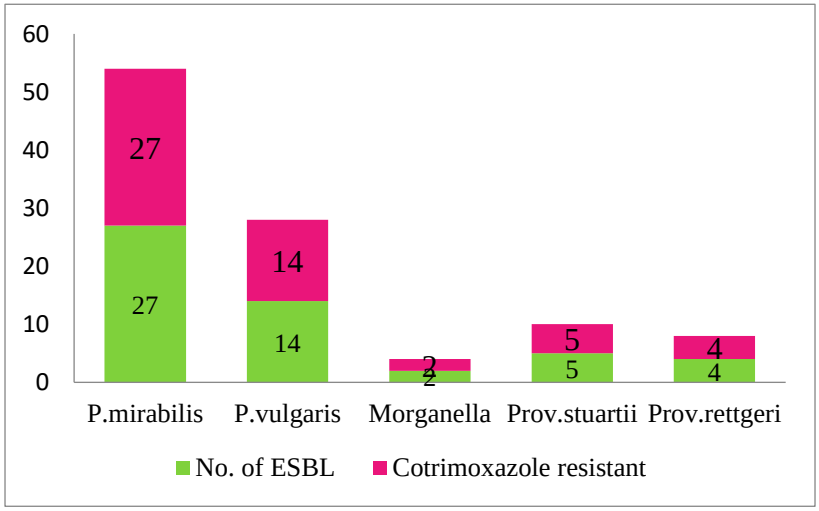


Figure 9. Number of ESBL producers resistant to cotrimoxazole

DISCUSSION

Tribe *Proteeae* are widely distributed in the environment as saprophytes. Within this tribe, the most common human pathogens belong to the genus *Proteus* [11]. Members of this genus can also be called as nosocomial pathogens and can cause infections of the respiratory tract, and more commonly wound infections. Mostly they are associated with urinary tract infections following catheterisation especially in the patients who have structural or functional abnormalities. 90 % of *Proteus* infections are due to *Proteus mirabilis.* Of the clinical samples collected, the maximum number of *Proteeae* isolates were from the exudate specimens (wound swabs, pus, abdominal abscess), accounting for 48% of all the specimens (Figure no. 1). Of the total of 145 isolates, 64 (45%) were found to be *P.mirabilis*, 48(33%) were *P.vulgaris*, 19 (13%) were *Morganella morganii*, 5 (3%) were *Providencia stuartii* , 9 (6%) were *Providencia rettgeri* (Figure no. 3a). This study correlates with the study of Feglo et al where *P.mirabilis* was the commonest isolate-61%.[12] But in a study of Reslinski 2005, *Proteus* species were commonly found in urine than other clinical specimens [13].

The number of *Proteus mirabilis* isolates from both exudate and respiratory samples was 39 and 9 respectively. The other common species isolated was *P.vulgaris* which was more common in urine than exudate samples (Figure no.3b) in the results which correlates with the study of Pandey et al[18].

In this study *P.mirabilis* isolates were 97% sensitive to Imipenem followed by 84% to piperacillin-tazobactam, 75% to meropenem, 75% ciprofloxacin and amikacin, 66% to cefipime. Although *P.mirabilis* were sensitive to most of the antibiotics, low sensitivity were observed to cotrimoxazole 42% and ampicillin 50% (Table no.1). In 1979 Chow et al in his study, has reported a multiple resistant P.mirabilis strain which was resistant to ampicillin along with cotrimoxazole, cephalothin, and aminoglycosides[14].

Most of the *P.vulgaris* isolates were sensitive to all antibiotics except ampicillin, cefazolin, and cefuroxime to which these organisms were intrinsically resistant. (Table no.1) Low sensitivity was noted only in cotrimoxazole 46%. This study correlates with the study by England Wales 2003 where multiple resistance patterns was not observed [15].

Providencia species exhibited more resistance to antibiotics than the other genera. It was observed that *Prov.stuartii* and *Prov.rettgeri* showed only 60% and 89% sensitivity to imipenem respectively. *Prov.stuartii* showed 100% resistance to third generation cephalosporin and only 20% of the isolates were sensitive to aminoglycosides, cotrimoxazole and meropenem (Table no.1). Similar results were observed in a study by Vinoth et al, which showed that *Providencia* species were 90% resistant to third generation cephalosporins,70% amikacin[16].

Most of the *Morganella* species were sensitive to all the antibiotics. The antibiotic resistance among the *Proteeae* species may be due to the indiscriminate use of antibiotics which provides selective pressure and thereby leading to development of resistant organisms (Levy 1999)[17].The *Proteeae* isolates that were resistant to third generation cephalosporins were tested for the production of ESBL by double disc synergy test. The picture shown in figure no.5 confirms ESBL production. According to figure no.6 of this study, out of total 145 Tribe *Proteeae* isolated, 52 isolates (36%) were extended spectrum beta lactamase producers i.e 27 (42%) out of 64 *P.mirabilis*, 14(29%) out of 48 *P.vulgaris*, 2 (11%) out of 19 *Morganella* and 9(64%) out of 14 *Providencia* species. In a study of Jitendra Kumar Pandey 2013, 48.8% of the isolates were ESBL producers which were found to correlate with the results of this study [18]. Whereas in a study by Joanna et al, among the 50 *P.mirabilis* strains isolated 11 (22%) were ESBL producers which is slightly lower from this study[19]. ESBL production was found to be more common among the *Providencia* species than Proteus and *Morganella* whereas in a study by Feglo et al ESBL production among the *Proteus* species was more 77%. [12].

Figure No.8 demonstrates the percentage of carbapenemase producers among the ESBL producing isolates. The picture shown in figure no.7 is positive for Modified Hodge Test. Among the 52 ESBL producers, 6(11.5%) were found to be resistant to carbapenems by disk diffusion method. These 6 isolates were confirmed by Modified Hodge test for carbapenemase production, according to CLSI guidelines [8]. Among these 6 isolates *Prov.stuartii* and *P.vulgari* isolates were 2 each, 1 was *Prov.rettgeri,* and 1 *P.mirabilis.*(Table no.2). A study in China showed that *P. mirabilis* from pleural drainage

fluid was resistant to carbapenems and showed the presence of the gene bla_{KPC}[20] .A study in Brazil by Ana Paul et al isolated a carbapenem resistant *Providencia rettgeri* in which bla NDM-1 was detected[21]. Therefore various genes responsible for this carbapenem resistance among the *Proteus* species is also emerging like ESBL. Therefore the results of this study show that the most effective antibiotic against the *Proteus* species was Imipenem followed by ciprofloxacin and amikacin. Also it has been noted that all the 52 ESBL producing isolates showed resistance to cotrimoxazole (Figure no. 9). The high antibiotic resistance may lead to increase in infections and the spread of the resistance to other individuals.

CONCLUSION

This study enumerates the different species of Tribe *Proteeae;* the prevalence of ESBL producing *Proteeae* isolates was significant 36% and the prevalence of Carbapenemase production was found to be 11.5%. These resistant strains not only cause serious infections among the individuals but also act as potential reservoirs of drug resistance genes which can be transferred to other organisms. The high level of ESBL production and the carbapenemase production are the indication of the resistance which has been recorded in many studies. Therefore, routine detection of ESBL and Carbapenemase detection should be carried out in each laboratory by standard methods recommended by Clinical Laboratory standard Institute (CLSI). This is important to control the spread of infections, to control the multi drug resistance, and to initiate proper therapeutic approach. And also indiscriminate use of the antibiotics should be avoided as the treatment options are limited in multidrug resistant strains.

REFERENCES

1) Stamm, W.E., and Norrby S.R. Urinary Tract Infections: Disease Panorama and Challenges. Clinical Infectious Disease. (2001) 183 (S1-S4)
2) Coker, C.; Barkare, O. O. and H. L. T. Mobley (2000): H-NS is a repressor of the *Pr. mirabilis* urease transcriptional activator gene ureR. J. Bacteriol. 128 (9): 2649-2553.
3) Jacobsen S.M., Stickler D.J., Mobley H.L.T. and Shirtliff M.E. (2008). Complicated Catheter- Associated Urinary Tract Infections due to *Escherichia coli* and *Proteus mirabilis*. *Clinical Microbiology Reviews*, 21(1): 26-59.
4) Luzzaro F., Perilli M., Amicosante G., Lombardi G., Belloni R., Zollo A., et al. 2000. Properties of multidrug- resistant, ESBL- producing *Proteus mirabilis* isolates and possible role of β-lactam/β-lactamase inhibitor combinations. Int J Antimicrob Agents 17: 131-135.
5) D'Andrea M.M., Literacka E., Zioga A., Giani T., Baraniak A., Fiett J., et al . Evolution and Spread of a Multidrug resistant *Proteus mirabilis* clone with chromosomal AmpC- Type Cephalosporinases in Europe. 10.1128 / *Antimicrob.Agents Chemother*. 2011 , 55 (6):2735-42.
6) Bush K, Jacoby GA, Medeiros A A. A functional classification scheme for beta lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995;39:1211-33.
7) Clinical and Laboratory Standards Institute (CLSI) 2013. Performance standards for antimicrobial susceptibiliy testing. M100-S20. CLSI, Wayne, PA.
8) De Champs C., Bonnet R., Sirot D., Chanal C., and Sirot J., Clinical relevance of *Pr.mirabilis* in hospital patients: A two year survey. J.Antimicrob Chemother. (2000). 45 (4): 537-539.
9) Feglo P.K., Gbedema S.Y., Quay S.N.A., Adu-Sarkodie Y., and Opoku-Okrah C., Occurence, species distribution and antibiotic 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.
10) Chow A W, Taylor P R, Yoshikawa T T, Guze L B. A nosocomial outbreak of infections due to multiple resistant *Proteus mirabilis*: Role of intestinal colonisation as a major reservoir. *J Infect Dis*. (1979);139(6) :621-627.
11) England,Wales and Northern Ireland:2003 *Proteus spp*, *Morganella morganii*, and *Providencia* spp bacteremias, *CDR Weekly* Vol14 (8), 19 February 2004
12) Vinoth J., Begum S E., Kumar S. R., Ramesh S. Phenotypic detection and antibiogram of Amp C Beta Lactamases Producing Tribe *Proteeae* In a Tertiary Care Hospital. *Asian J Pharm Clin Res, (2012) Vol 5(4):*180-182
13) Pandey J.K., Narayanan A., Tyag i S., Prevalence of *Proteus* species in clinical samples, antibiotic sensitivity pattern and ESBL production. *Int.J.Curr.Microbiol.App.Sci* (2013) 2(10): 253-261
14) SHENG Zi-ke, LI Jun-jie, SHENG Guo-ping, SHENG Ji-fang and LI Lan-juan., Emergence of carbapenemase producing *P.mirabilis* in Hangzhou, China. *Chin Med J 2010;123(18):2568-2570.*