A study on the antibiotic susceptibility pattern of 

Proteus spp among various samples.

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

Key words: Proteus, ESBL, susceptibility.

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

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

MATERIALS AND METHODS
This study was conducted in the microbiology department, Meenakshi Medical College and Research Institute at Kanchipuram during the period of February 2013 to April 2014. Total of 121 clinical isolates of Proteus spp were collected from wound, pus, sputum, urine, blood and body fluids.
Demographic data were recorded prior to sample collection from patients. Ethical clearance was obtained from institutional ethical committee. All the samples were collected with aseptic precautions and inoculated onto nutrient broth, blood agar, MacConkey agar and incubated at 37°C, overnight. The colonies were tested for the standard biochemical reactions for Proteus spp. The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion technique with commercially available disc (Hi media) on Muller Hinton agar. E.coli ATCC 25922 was used as control and results were interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines. Those strains showed resistance to 3rd generation cephalosporins were subjected to ESBL detection methods using combined disc diffusion test. (CLSI-2013)

ESBL Screening (CLSI-2013)
Screening of Proteus spp. for ESBL production was performed according to the procedures as recommended by the CLSI, using indicator cephalosporins, ceftriaxone (30 μg), ceftazidime (30 μg), and cefotaxime (30 μg). Isolates exhibiting zone size ≤ 25 mm with ceftriaxone ≤ 22 mm for ceftazidime and ≤ 27 mm with cefotaxime were considered as ESBL producer.

Phenotypic Confirmatory Test for ESBL: (Combined Disc Diffusion Method) (CLSI-2013)
0.5 McFarland turbidity standard suspension was made from the colonies of Proteus spp. isolate. Using this inoculum, lawn culture was made on Muller Hinton Agar (MHA) plate. Discs of Ceftazidime and Ceftazidime + Clavulanic acid (30 mcg/10 mcg) were placed aseptically on the surface of MHA. The distance of 15 mm was kept between the disc and overnight incubation was done at 37°C. An increase of ≥ 5 mm in zone diameter of Ceftazidime + Clavulanic acid in comparison to the zone diameter of Ceftazidime alone confirmed the ESBL production by the organism.

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

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

In this present study, the prevalence rate of Proteus among various clinical specimens is 3.04%. The same has been reported with Bahashwan et al (3%)5. Slightly lower prevalence rate 1.12%, has been observed by Pandey JK etal, whereas Jaber MH etal reported a high prevalence of 28.75%, in his study.10, 2

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

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

In this study, Proteus spp were equally isolated (44.6%) from pus (Table-1). Feglo et al., 13 and Leulmi et al 14 observed maximum isolates in pus samples. Male (63.6%) were commonly affected than female (36.4%) (Table-2). UTI due to Proteus spp is more common in males. It is concordant with the studies of Bahashwan et al and Nita Pal et al. 9, 1 Male prepondarence is mostly due to frequent increased outdoor activities and exposure to environment and infectious agents.

In our study, 3 Proteus species P.mirabilis, P.vulgaris & P.penneri were isolated at the rate of 66.9%, 31.5% & 1.6% respectively. (Figure-1). P.mirabilis was the predominant isolates in variuos reports. 6, 10, 14, 15, 16. P.mirabilis causes 90% of Proteus infections such as meningitis,empyema, osteomyelitis and gastroenteritis. Also, it is implicated in nosocomial infections of urinary tract(46%),surgical wounds(24%) and respiratory tract (30%). It is believed that the most common cause of infections related to kidney stone, is the most
common serious complication of unresolved and recurrent bacteriuria. So early isolation and speciation of *Proteus* and monitoring the antibiogram pattern will help to prevent the complications. Imipenem was the most effective drug of resistant strains of gram negative bacilli. In our study, results of antimicrobial susceptibility test revealed that Imipenem was the most effective antibiotic against *Proteus* spp with the sensitivity rate of 99.1%, which is followed by Piperacillin-Tazobactam with the sensitivity rate of 92.5% (Table-3). Similar pattern were observed with Nita Pal et al. & Shenoy et al. Though imipenem was found to be unaffected by the enzymes in our study, the variation in the resistance reports could be due to the study environment.

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

All the isolates showed lower sensitivity rate of 19.8 % & 8.26% to Amoxyclav & Ampicillin respectively. It is in accordance with the studies of Nita Pal et al & Vinoth et al. Attention should be focused on the decreasing trend of susceptibility to this group of drugs because prescription of these antibiotics to *Proteus* infections will end up with multi drug resistance (MDR), extended drug resistance (XDR) and pan drug resistance (PDR) which is worrisome. MDR is pervasive and emerging clinical problem, which causes significant morbidity, mortality and increased economical burden which stems from the inappropriate, excessive use of antibiotics.

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

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

The ESBL resistance patterns of the isolates are indicators of an increase in the resistance menace reported by earlier studies. Emergence of infections caused by ESBL, MBL, MDR, XDR & PDR *Proteus* is alarming which creates serious health problem resulting in an enormous burden in health care setup and cost. Reducing susceptibility of *Proteus* spp and the emerging resistance illustrated in this group need for routine susceptibility tests. This study is therefore a step towards the generation of national data on the prevalence of antimicrobial resistance pathogens.

**Limitations of this study**

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

**CONCLUSION**

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

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Number (n=121)</th>
<th>Percentage (%)</th>
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</thead>
<tbody>
<tr>
<td>Urine</td>
<td>61</td>
<td>50.4</td>
</tr>
<tr>
<td>Pus</td>
<td>54</td>
<td>44.6</td>
</tr>
<tr>
<td>Blood</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>Sputum</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>1</td>
<td>0.8</td>
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Table-2
Sex distribution of Proteus spp

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number (n=121)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>77</td>
<td>63.6</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>36.4</td>
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</tbody>
</table>

Figure-1
SPECIES DISTRIBUTION (n=121)

Table-3
Antibiotic susceptibility pattern of Proteus spp.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Number (n=121)</th>
<th>Percentage (%)</th>
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</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>120</td>
<td>99.1</td>
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<tr>
<td>Piperacillin tazobactum</td>
<td>112</td>
<td>92.5</td>
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<td>Ofloxacin</td>
<td>74</td>
<td>61.1</td>
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<tr>
<td>Amikacin</td>
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<td>47.1</td>
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<td>Ciprofloxacin</td>
<td>52</td>
<td>42.9</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>45</td>
<td>37.1</td>
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<td>Ceftriaxone</td>
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<td>33.8</td>
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<tr>
<td>Doxycycline</td>
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<td>28.9</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>35</td>
<td>28.9</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>32</td>
<td>26.4</td>
</tr>
<tr>
<td>Amoxyclav</td>
<td>24</td>
<td>19.8</td>
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<tr>
<td>Ampicillin</td>
<td>10</td>
<td>8.26</td>
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REFERENCES


