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Research Article

PHENOTYPIC DETECTION AND ANTIBIOGRAM OF AMPC BETA-LACTAMASES PRODUCING TRIBE PROTEEAE IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: AmpC beta-lactamases are clinically significant cephalosporinases produced in several Enterobacteriaceae, which confer resistance to cephamycins, penicillins, aminoglycosides and β -lactam- β-lactamase inhibitor combinations.
Objective: To estimate the prevalence of AmpC β-lactamases producing tribe Proteeae by phenotypic detection methods at a tertiary care hospital in south India.
Materials and Methods: The study was carried out with a total of 97 clinical isolates comprising of *Proteus vulgaris* (N=41), *Proteus mirabilis* (N=46), *Providencia stuartii* (N=7) and *Providencia rettgeri* (N=3). The susceptibility of the clinical isolates to standard antibiotics was tested by Kirby-Bauer disc diffusion technique. The isolates were further screened for the production of AmpC β-lactamases by AmpC disc test and Cefoxitin Hodge Test.
Results: 57% of tribe Proteeae isolates was shown to produce AmpC β-lactamases. The enzyme was essentially detected in *P. vulgaris* 52% (N=29), *P. mirabilis* 35% (N=19), *P. stuartii* 11% (N=6) and *P. rettgeri* 3.5% (N=2). The clinical isolates were resistant to cephamycins, penicillins, and β -lactam- β-lactamase inhibitor combinations and highly sensitive to carbapenems namely imipenem and meropenem.
Conclusions: The findings of the present study revealed the distribution of AmpC β-lactamases in *Proteus* spp. and *Providencia* spp. of genera tribe Proteeae in the hospital. The phenotypic methods showed excellent specificity, which can be employed for screening AmpC β-lactamases producing microbes in the clinical specimens. These methods can be recommended in various clinical laboratories, where the facilities are limited to evade severe nosocomial infections.

Keywords: AmpC beta-lactamases, clinical isolates, *Proteus* spp., *Providencia* spp., tribe Proteeae

INTRODUCTION

AmpC beta-lactamases are clinically important cephalosporinases produced in several Enterobacteriaceae, which confer resistance to cephamycins, penicillins, and β -lactam- β-lactamase inhibitor combinations[1]. Microbes acquire these enzymes by horizontal gene transfer of the plasmid DNA. Persistent treatments with standard antibiotics lead to the genesis of these enzymes worldwide in diverse species of Enterobacteriaceae, such as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, *Salmonella* spp. and *Proteus mirabilis*[2-10]. Plasmid-mediated AmpC beta-lactamase are constitutively expressed in Enterobacteriaceae and are relatively rare [11,12]. Several AmpC enzyme producing bacteria are retrieved from hospitalized patients after several days of admission in the hospital. Although it has been over a period of decade since plasmid-mediated AmpC beta-lactamases were discovered, most clinical laboratories remain ignorant of their clinical consequences[13]. Consequently microorganisms producing these enzymes are concealed and are primarily liable for various nosocomial infections in hospitals.

The genera *Proteus* and *Providencia,* are related members of family Enterobacteriaceae grouped under the tribe Proteeae. These organisms in general, are common urinary tract pathogens, associated with urinary tract infections (UTIs) [14,15]. *P. mirabilis* has higher incidence in UTIs, yet are typically uncomplicated due to its sensitivity to universally prescribed antibiotics, whereas *P. vulgaris* confer higher resistance to common antibiotics prescribed for UTIs[16]. Among *Providencia* spp., *P. stuartii* is the most widespread species isolated from clinical specimens while *P. rettgeri* are infrequently diagnosed and is relatively rare in urinary tract infections[17]. *P. rettgeri* and *P. stuartii* are intrinsically resistant to diverse antibiotics including gentamicin, first generation cephalosporins and ampicillin. The objective of this study is to estimate the presence of AmpC beta-lactamases in *Proteus* spp. and *Providencia* spp. at a tertiary care hospital using customary phenotypic detection methods.

MATERIALS AND METHODS

Sample collection

The clinical isolates for the study were obtained from Sri Ramachandra medical centre, a tertiary care center at Porur in Chennai for a period of three months between December 2010 and February 2011. The clinical isolates were generous gifts from the Central laboratory, Sri Ramachandra medical centre.

Biochemical tests

All biochemical tests were done according to standard procedures[18].

Antibiogram

The following antibiotics were tested by disc diffusion method [19,20], Ampicillin (10 µg), amikacin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), co-trimaxazole (1.25/23.75 µg), tobramycin (10 µg), imipenem (10 µg), meropenem (10 µg), cefaperazone-sulbactum (75/30 µg), tazobactum-piperacillin (10/100 µg) and norfloxacin (10 µg) (Himedia). The isolates were swabbed on Mueller-Hinton agar and the antibiotic discs were placed on the agar surfaces. The petri plates were incubated for 24 hours at 37° C. The sensitivity to first line and second line antibiotics were interpreted after 24 hours incubation as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

AmpC disc test

The test is based on ability of tris-EDTA to permeabilize a bacterial cell and discharge β-lactamases into the external environment. The surface of a Mueller-Hinton agar plate was inoculated with a lawn culture of cefoxitin-susceptible *E. coli* ATCC 25922. A 30 µg cefoxitin disc was placed on the surface of the agar. A sterile plain disc containing test organism was placed adjacent to the cefoxitin disc roughly touching it, with the inoculated disc, face in contact with the agar surface. The plates were incubated overnight at 37°C. The plates were scrutinized for either an serration or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result) or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result) [21].

Cefoxitin Hodge Test

The surfaces of MacConkey agar plates were inoculated with a lawn of the indicator strain, ATCC *E. coli* 25922, according to the CLSI disk-diffusion method. After drying of the lawn culture, a test strain was heavily streaked from the center of the plate to the periphery and a cefoxitin disk was placed at the center. The plates were

incubated overnight at 37°C. The presence of explicit growth of the indicator strain in the inhibition zone along with the test strain was interpreted as positive [22].

RESULTS

Based on biochemical parameters, the isolates were grouped as *P. mirabilus, P. vulgaris, P. rettgeri* and *P. stuartii* (Table 1). The isolates were tested for antibiotic susceptibility and AmpC production by AmpC disc test and Cefoxitin Hodge test (Figure 1). The Antibiogram of *Proteus* spp. isolates is depicted in Figure 2. Among the β-lactams, maximum resistance was observed with ampicillin (85 %). With the cephalosporins, 85 % of the isolates were resistant to cefoxitin, 41 % to cephotaxime and 38 % to ceftazidime. 24 % of the isolates were resistance to norfloxacin. With aminoglycosides, privileged fraction of the isolates were resistant to tobramycin (21 %) followed by amikacin (9 %). 36 % of the isolates were resistant to co-trimaxazole. With β-lactam - β-lactam inhibitor combinations, 2% of the isolates were resistant to cefaperazone-sulbactum and tazobactum piperacillin respectively. The *Proteus* spp. isolates were highly sensitive to carbapenems namely, imipenem and meropenem (Figure 2).

Table 1: Specimen wise distribution of tribe Proteeae isolates

Clinical isolates	Exudates	Blood	Urine	Total
P. mirabilis	25	1	20	46
P. vulgaris	21	0	20	41
P. rettgeri	0	0	3	3
P. stuartii	4	0	3	7
Total	50	1	46	97

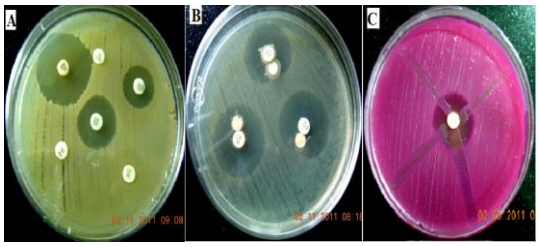

Figure 1: Phenotypic detection of AmpC beta-lactamase producers. A: Antibiotic susceptibility testing, B: AmpC disc test, and C: Cefoxitin Hodge Test

Among the tribe Proteeae isolates, the genus *Providencia* was the most resistant organisms contributed by the highly resistant *P. stuartii*. *P. stuartii* was unanimously resistant to ampicillin and first generation cephalosporins and 90 % were resistant to third generation cephalosporins (Figure 3). Among aminoglycosides, 90% of *P. stuartii* were resistant to tobramycin and 70% to amikacin. 40% of the isolates were resistant to cefaperazone-sulbactum and tazobactum piperacillin. With carbapenems, only 30 % of the clinical isolates were resistant. 70% of *Providencia* spp. isolates were resistant to norfloxacin and co-trimaxazole.

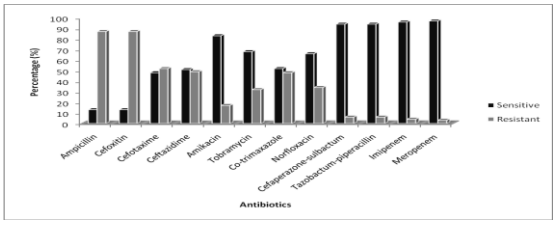

Figure 2: Antibiogram of *Proteus* spp

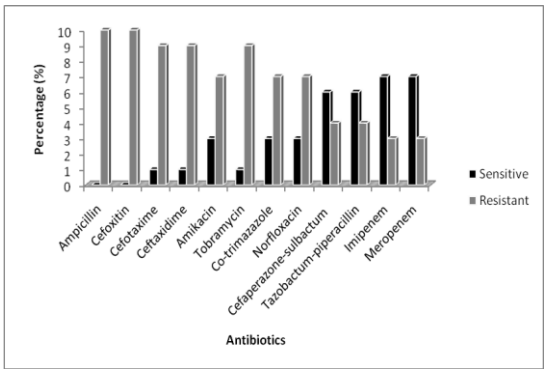

Figure 3: Antibiogram of *Providencia* spp.

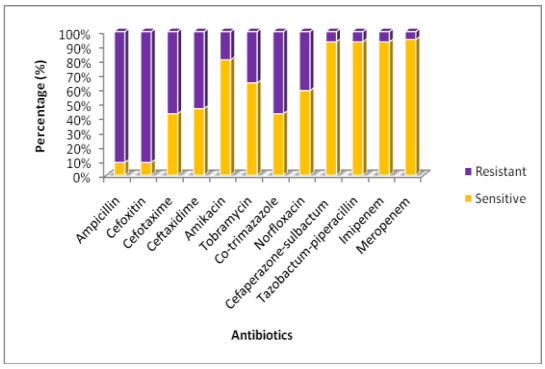

Figure 4: Antibiogram of AmpC beta-lactamase producers

The 56 isolates of *Proteus* spp. and *Providencia* spp were shown to produce AmpC beta-lactamases (Figure 4). 91 % were resistant to both ampicillin and cefoxitin, 57 % and 53 % of AmpC producers in tribe Proteeae were resistant to cephotaxime and ceftazidime respectively. 93 % of the isolates were vulnerable to both the β-lactamase- β-lactamase inhibitor combinations. Imipenem and meropenem were exceedingly effectual against AmpC producers.

DISCUSSION

Tribe Proteeae belong to Enterobacteriaceae family of Gram-negative bacilli which include *Proteus* spp., *Providencia* spp. and *Morganella* spp.. *P. mirabilis* records 90% of *Proteus* infections and is regarded as a community-acquired infection whereas *Proteus vulgaris* and *P. penneri* are usually isolated from individuals in long-term care facilities and hospitals [23.24.25]. *P. mirabilis* is the most susceptible amid the group. Our result is contemporaneous with the previous reports with 35 % of *P. mirabilis*, being resistant to ampicillin and first generation cephalosporin. AmpC β-lactamase producing *P. mirabilis* were sensitive to carbapenems. *P. vulgaris* was highly sensitive to ceftazidime, meropenem, cefaperazone-sulbactum and tazobactum-piperacillin. In the present study, 52 % *P. vulgaris* isolates were shown to produce AmpC beta-lactamases. *P. stuartii* is yet another species in tribe Proteeae known to possess universal resistance to most of the antibiotics. They are resistant to penicillin, cephalosporins, and the other cephamycins, but are sensitive to ceftazidime, cephotaxime imipenem. Our study reports that the *Providencia* isolates possessed inherent resistant to ampicillin and first generation cephalosporins and β -lactam- β-lactamase inhibitors.

Despite the discovery of AmpC β-lactamases a decade ago, there remains a low level of consciousness of their significance. Several clinical laboratories have problems in detecting AmpC β-lactamases in the clinical isolates. Uncertainty subsists about the importance of these resistance mechanisms and appropriate coverage. Failure to perceive these enzymes have contributed to their unrestrained spread and therapeutic letdown. Hodge test and Tris-EDTA (AmpC) disc test are golden standard for the detection of Amp C enzymes. In the present study, the Hodge test showed 60 of the isolates to be positive and Amp C disk test showed 56 to be positive. 52 AmpC producers were identified by both the methods. The Hodge test

showed good number of isolates to be positive, while Tris -EDTA test showed better and consistent results than Hodge test. In the present study, AmpC β -lactamases was detected essentially in *P. vulgaris* 52% (N=29) followed by *P. mirabilis* 35% (N=19), *P. stuartii* 11% (N=6) and *P. rettgeri* 3.5% (N=2).

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