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Research Article

Susceptibility Trends of Pseudomonas Species from Ocular Infections at a Tertiary Care Hospital, Hyderabad

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Abstract: The choice of antibiotics to treat Pseudomonas infections of eye is challenging to ophthalmologist, considering the increase in prevalence of antibiotic resistant isolates. With the increase in occurrence and types of multiple β-lactamase enzymes, early detection is crucial, the benefits of which include implementation of proper antibiotic therapy and infection control policy. The present study was conducted for the isolation, identification of Pseudomonas species & its antibiotic susceptibility patterns in ocular infections with special reference to ESBL, MBL, and AMP-C beta lactamase detection. The present study was carried out at Department of Microbiology, Sarojini Devi Eye Hospital, Hyderabad, from March 2014 to August 2014. ESBL detection was done by combined disc test (CDT) & double disc synergy test (DDST). MBL detection was done by Ceftazidime-EDTA CDT and Ceftazidime-EDTA DDST. Amp-C detection was done by Disc antagonism test &AmpC disc test. Out of the 20 Pseudomonas isolated, 4(20%) were ESBL producers, 10(50%) were MBL producers, and 1 isolate (5%) was AmpC producer. Most sensitive antibiotic was Cefotaxime (100%), Amikacin (60%) followed by Ciprofloxacin &Gatifloxacin (50%) each. The present study emphasizes high prevalence of Pseudomonas producing beta-lactamase enzymes, creating therapeutic challenge for Clinicians and Microbiologists. Hence, routine surveillance of antibiotic resistance is required in the hospital.
Keywords: Pseudomonas, ocular, ESBL, MBL, AMP-C, antibiotic susceptibility.

INTRODUCTION

Analogous to many other infections, Indian population are vulnerable to infections of the eye by virtue of subtropical climate. Even what may be considered a minor infection elsewhere in the body, can be fatal to the eye in terms of visual compromise. Thus, eye care forms one of the major commitments among the medical fraternity in India [1]. Many opportunistic pathogenic agents are increasingly encountered in ocular infections. It is observed that empirical antibiotics are resistant as per culture report [2]. Hence to guide ophthalmologist to select proper antibiotic empirically, susceptibility trends are detected. Such studies are of great value to ophthalmologist who has to select first line antibiotic treatment [3].

Pseudomonas aeruginosa can produce all major classes of β-lactamase (A, C, D) and also metallo beta lactamases (class B)[4]. At present Clinical and Laboratory Standards Institute (CLSI) guidelines do not describe any method for detection of these enzymes in P.aeruginosa[5]. This study was undertaken to detect ESBL, MBL and AmpC ß lactamases producing Pseudomonas species by phenotypic methods from eye samples, & to provide an early, rapid and effective phenotypic method for identifying them.

MATERIALS AND METHODS

The present study was carried out at Department of Microbiology, Sarojini Devi Eye Hospital, a tertiary care centre, Hyderabad, from March 2014 to August 2014.Pseudomonas species isolated from patients of either sex & of all age groups during the six month period, with ocular infections, diagnosed by an ophthalmologist, were included in the study. All other bacterial & fungal clinical isolates other than Pseudomonas were excluded from the study. The Pseudomonas species confirmed by biochemical reactions were subjected to antibiotic susceptibility testing by Kirby - Bauer disc diffusion technique on Mueller Hinton agar according to CLSI guidelines. The antibiotic discs used were Cefotaxim, Ceftazidime, Cefazolin, Ciprofloxacin, Gentamicin, Amikacin, Tobramycin, Moxifloxacin, Gatifloxacin, Chloramphenicol, Pseudomonas aeruginosa ATCC 27853 was used as a control strain.

DETECTION OF ESBL

1. Disc diffusion test / combined disc test [8]

Test organism was inoculated with standard inoculum (0.5 McFarland) to form a lawn culture on to Mueller Hinton agar (MHA) plate. Ceftazidime (30µg) & Ceftazidime/Clavulanic acid (30µg/10µg) discs were placed on MHA plate and was incubated overnight at 37^{o}C. An increase in the zone diameter by > 5mm of Ceftazidime/Clavulanic acid when compared to Ceftazidime alone was considered as an ESBL producer.

2. Double Disc synergy test: [9]

Test organism was inoculated with standard inoculum (0.5 McFarland) to form a lawn culture on to Mueller Hinton agar plate. 30 µg discs of each third generation cephalosporin antibiotics - Cefotaxime and Ceftazidime, were placed on MHA plate at a distance of 15mm center to center from Amoxyclav disc (Amoxycillin / Clavulanic acid - 20µg/10µg) and were incubated overnight at 37^{o}C. Increase in the inhibition zone of any one of the third generation antibiotic disc towards Amoxyclav disc was considered as an ESBL producer.

DETECTION OF MBL

Phenotypic detection for MBL production was done by following methods.

1. Combined disc test
2. Double disc synergy test

EDTA solution preparation:

A 0.5 M Ethylene diamine tetra acetic acid (EDTA) solution was prepared by dissolving 186.1 g of disodium EDTA.2H_2O in 1000 ml of distilled water and adjusted it to pH of 8.0 by adding Sodium hydroxide (NaOH). The mixture was sterilized by autoclaving. 10µl of 0.5 M EDTA solution was used each time. It was stored in refrigerator at 4^{0}C in airtight vials without significant loss of activity for at least 12 weeks [10].

1. CAZ-EDTA combined disc test [21]

Test organism was inoculated on to Mueller Hinton agar plate .Two 30µg CAZ discs were placed on the plate, and appropriate amount of 10 µL of EDTA solution was added to one of them to obtain the desired concentration (750 µg).The inhibition zones of the CAZ and CAZ-EDTA discs were compared after overnight incubation at 37°C. If the increase in inhibition zone with the CAZ and EDTA disc was ≥ 7 mm than the CAZ disc alone, it was considered as MBL positive.

2. CAZ-EDTA double disc synergy test [11]

Test organisms were inoculated on to plates with Mueller Hinton agar. CAZ (30 µg) disc was placed 20 mm centre to centre from a blank disc containing 10 µL of 0.5M EDTA (750 µg) & was incubated overnight at 37°C. Enhancement of the zone of inhibition in the area between CAZ and the EDTA disc in comparison with the zone of inhibition on the far side of the CAZ disc was interpreted as a positive result.

DETECTION OF AMP C β LACTAMASES

Detection for AmpC β-lactamase production was performed by using Cefoxitin (30 µg) disc which antagonizes beta-lactamase via induction of drug-inactivating β-lactamase. The isolates were subjected to disc antagonism test for inducible AmpC enzyme, and AmpC disc test for the detection of plasmid AmpC β-lactamases [12].

1. Disc Antagonism Test [12]

In this test, lawn culture of test isolate (0.5Mc Farland) was put over Mueller-Hinton agar plate (MHA). Ceftazidime (30µg) & cefotaxime (30µg) discs were placed 20 mm apart centre to centre from Cefoxitin (30µg) disk. Plates were incubated for 18-24 hours at 37°C. AmpC β-lactamase inducibility was recognized by isolates showing blunting of Ceftazidime or cefotaxime zone of inhibition, adjacent to cefoxitindisc and was considered positive.

2. AmpC disc test [13]

A lawn culture of E. coli ATCC strain 25922 was prepared on MHA plate. Sterile discs (6 mm) were moistened with sterile saline (20µl) and inoculated with several colonies of test organisms, were placed beside a cefoxitin disc (almost touching) on the MHA plate. The plates were incubated for 18-24 hours at 37°C. Flattening or indentation of the cefoxitin zone in the vicinity of the test disc was considered positive. A negative test had an undistorted zone.

RESULTS

Out of 280 bacterial isolates from ocular infections, Staphylococcus epidermidis (74%) were highest, followed by Staphylococcus aureus (12.85%) and Pseudomonas isolates were 7.14%.Of the 20 isolates,18 (90%) were Pseudomonas aeruginosa and 2(10%) were Pseudomonas alcaligenes. A total of 20 Pseudomonas isolated from various ocular samples were subjected to antibiotic susceptibility testing and screened for ESBL, MBL and AmpC production. Antibiotic susceptibility pattern showed maximum sensitivity to Cefotaxime (100%), Amikacin (60%) followed by Ciprofloxacin& Gatifloxacin (50%) each.**(**Table1)

ESBL detection was done by CDT & DDST (Fig 1).Out of the 20 isolates, 4(20%) were ESBL producers. Of the 4 ESBL producers, 1 isolate was detected by CDT, 2 were detected by DDST and 1 was positive by both methods.MBL detection was done by CAZ-EDTA CDT and CAZ-EDTA DDST(Fig 3).Out of 20 isolates, 10(50%) were MBL producers. Of the 10 MBL producers, 9 were detected by CDT and 1 was detected by both CDT and DDST. Two isolates (10%)

were both ESBL and MBL producers (Fig 5). Of the 20 isolates, only one isolate was AmpC producer, detected by disk antagonism test (Fig 4). None of the isolates were positive by AmpC disk test (Fig 2).

Table–1: Antimicrobial susceptibility pattern of Pseudomonas isolates.

Antibiotics	Sensitive (%)	Resistant (%)
Chloramphenicol	7(35)	13(65)
Gentamicin	9 (45)	11 (55)
Tobramycin	9 (45)	11(55)
Ciprofloxacin	10(50)	10 (50)
Ofloxacin	8 (40)	12 (60)
Gatifloxacin	10 (50)	10 (50)
Morifloxacin	8 (40)	12 (60)
Cefazolin	8 (40)	12 (60)
Ceftazidime	8 (40)	12 (60)
Cefotaxime	20(100)	0(0)
Amikacin	12(60)	8 (40)

Majority of the Pseudomonas isolates were sensitive to Amikacin (60%), followed by Ciprofloxacin (50%), Gatifloxacin (50%). They were least sensitive to Chloromphenicol (35%).

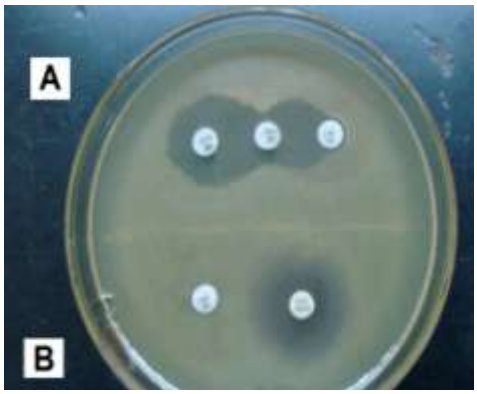

Fig1: ESBL producing P. aeruginosa A: DDST- Increase in zone diameter of CTX (L) and CAZ(R) towards AMC(C). B: CDT-CAC disc(R) zone >5mm than CAZ (L) alone.

Fig 2: Amp-C detection by Amp-c disc test Negative – Undistorted zone of Inhibition of CX (C) disc

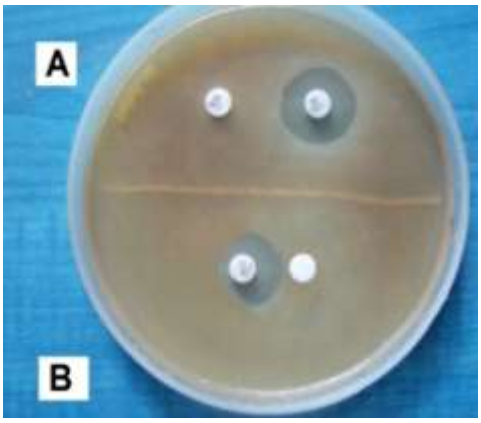

Fig 3: MBLproducing P. aeruginosa A: CAZ-EDTA CDT - CAZ-EDTA(R) >7mm than CAZ (L) Alone. B: CAZ – EDTA DDST - Increase in Zonediameter of CAZ (L) towards blank disc + EDTA (R). Brown pigment seen.

Fig4: Amp-C detection by disc antagonism test blunting of CTX (R) & CAZ (L) zone of Inhibition adjacent to CX (C) disc

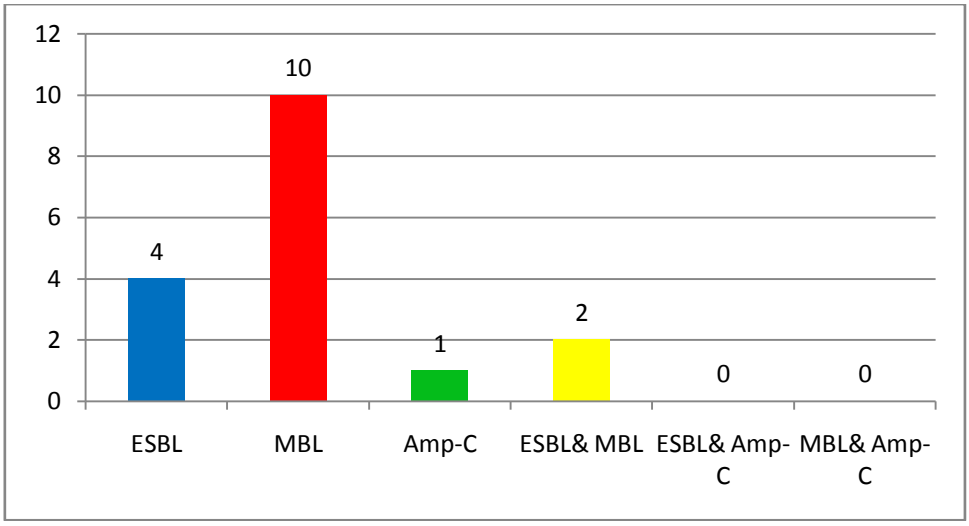

Fig 5:Graph showing types of ß lactamase produced by Pseudomonas.

DISCUSSION

Pseudomonas aeruginosa is well recognized as an ocular pathogen causing severe keratitis, corneoscleritis and endophthalmitis. Keratitis may result in corneal melting and perforation [14]. Infections caused by Pseudomonas aeruginosa are difficult to treat as the majority of isolates exhibit varying degrees of innate resistance. Acquired resistance is also reported by the production of ESBL, MBL & AmpC β-lactamases.

With the increase in occurrence and types of these multiple β-lactamase enzymes, early detection is crucial, the benefits of which include implementation of proper antibiotic therapy and infection control policy [5]. Results from the present study showed the presence of different classes of β-lactamase enzymes and indicate shifting trends of antibiotic susceptibility in case of Pseudomonas species.

In present study, cefotaxime showed 100% sensitivity. It is not available in topical preparation. It is used systemically only for endophthalmitis cases & severe perforated corneal ulcers. The Pseudomonas isolates in our study were more susceptible to topical antibiotics like amikacin (60%) followed by ciprofloxacin (50%) &gatifloxacin (50%). Chloramphenicol (35%) was the least sensitive antibiotic, antimicrobial susceptibility pattern of Pseudomonas in various studies in Table 2.

Table 2: Antimicrobial susceptibility pattern in various studies

Study series	Year	AK	TOB	GEN	C	CIP	OF	GAT	MO	CZ	CAZ	CTX
Bharathi et al [16]	2010	88	30	33	60	85	87	88	79	-	80	64
Ramesh et al [17]	2010	90	50	79	31	64.2	79	92	43	6	74	76
Mullasumaiya et al [18]	2012	88	50	63	63	88	88	88	-	-	75	75
Sunil Kumar et al [19]	2012	100	50	54.2	-	57.9	69.4	-	-	42	-	-
Kalia murty *et al.;* [15]	2013	89	73	89.7	40	82.9	73.5	73.5	82	-	-	-
Present study	2014	60	45	45	35	50	40	50	40	40	40	100

Ceftazidime is a third generation cephalosporin, used frequently for the treatment of infections caused by P. aeruginosa. However, the resistance to ceftazidime is increasing at an alarming

rate, complicating the clinical management of patients infected with such isolates [20]. In this study, a high level of resistance (60%) to ceftazidime was observed among the P. aeruginosa isolates.

Ceftazidime resistance is mainly mediated by production of β-lactamases such as ESBL, MBL and occasionally AmpC- β-lactamases. Besides production of various β-lactamases, other mechanisms such as the lack of drug penetration due to mutation in porins, loss of certain outer membrane proteins and efflux pumps can also contribute for resistance to β-lactams[20].

ESBL DETECTION

Extended-spectrum β-lactamases are enzymes that mediate resistance to extended-spectrum cephalosporins and monobactams. In the present study, 20 % of the Pseudomonas isolates were ESBL producers. Similarly, Umadevi *et al.;* [20] in 2011 & Sunilkumar *et al.;* [7] in 2012 reported 19.4% & 24% ESBL producers which are in accordance with our study.

MBL DETECTION

This variation in the prevalence of MBL producing P. aeruginosa in different places &studies **(**Table3) could be due to the variation in sample size studied or due to their differences in hygienic practices.

AMP-C DETECTION

In our study, about 5% of the Pseudomonas isolates were noted to produce AmpC β-lactamases. Umadevi *et al.;* [20] &Basak *et al.;* [4] reported 16.4% & 19.3 % respectively. Other studies from India [5, 12] reported high prevalence of 50% & 29.6% of AmpC production **(**Table4).

Table 3: MBL detection in various studies

Study series	Year	MBL (%)
S.Umadevi *et al.;* [20]	2011	65.7
Niravpandya *et al.;* [21]	2011	9.92
Sunil kumar et al [7]	2012	7
Paula regina *et al.;* [6]	2012	44.8
Ejikugwu *et al.;* [22]	2014	10
Present study	2014	50

Table 4: Amp-C detection in various studies

Study series	Year	AmpC (%)
Basak.S *et al.;* [4]	2009	19.3
Upadhyay *et al.;* [5]	2010	50
S.umadevi *et al.;* [20]	2011	16.4
Manojkumar *et al.;* [12]	2013	29.6
Present study	2014	5

CONCLUSION

The present study underlines the unique problem of ESBL; MBL & AMP-C mediated resistance, which has created a therapeutic challenge for Clinicians and Microbiologists. Detection of beta-lactamase production is of paramount importance both in hospital and community isolates because

- These strains are probably more prevalent than currently recognized.
- These enzymes constitute a serious threat to currently available antibiotics.
- Institutional outbreaks are increasing because of the selective pressure due to the in discriminate use of expanded spectrum cephalosporins and lapses in effective control measures.

Vigilance and timely recognition of infection with resistant bacteria and appropriate antibiotic therapy, is the only answer to the current multidrug resistant bacteria population. A routine surveillance of antibiotic resistance in the hospital is recommended. Bacterial strains resistant to most classes of antibiotics will continue to emerge unless in appropriate use of drugs is curtailed and continuous education of infection control practices is maintained.

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