Antibiotic sensitivity trends of pseudomonas endophthalmitis in a tertiary eye care center in South India: A 12-year retrospective study

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Purpose: To assess trends in antibiotic sensitivity of pseudomonas and compare multidrug resistance (MDR) between Pseudomonas endophthalmitis cases presenting in two consecutive 6-year time frames in a tertiary center in South India. **Methods:** This is a retrospective comparative series of all Pseudomonas endophthalmitis cases treated from June 2004 to May 2016. Microbiological culture results in all endophthalmitis patients were screened for pseudomonas. Positive cases in the initial 6 and final 6 years were compared for sensitivity to antibiotics and the proportion of MDR. MDR was defined as resistance to at least two different classes of antibiotics. **Results:** Pseudomonas accounted for 74 of 389 endophthalmitis cases (19%), 42 in initial 6 and 32 in final 6 years. Sensitivity to ciprofloxacin, ofloxacin, gatifloxacin, moxifloxacin, and ceftazidime was 85.7%, 82.9%, 76.5%, 76.9%, 88.1% up to 2010 which reduced to 75%, 59.4%, 68.8%, 56.3%, 56.3%, respectively, after 2010, being significant for ofloxacin ($P = 0.0349$) and ceftazidime ($P = 0.0028$). Susceptibility to amikacin, gentamicin, and tobramycin changed non-significantly from 83.3%, 43.9%, 47.6% to 71.9%, 61.3%, 61.3%, respectively. Twenty of 74 cases (27%) were MDR with 16.7% in first 6 years versus 40.6% in final 6 years. Postoperative MDR cases rose from 10.3% to 50% ($P = 0.0048$). **Conclusion:** This study shows rising resistance of Pseudomonas to fluoroquinolones, amikacin, and ceftazidime in endophthalmitis. MDR also showed an upward trend, particularly in postsurgical cases.

Key words: Antibiotic sensitivity, multi-drug resistance, pseudomonas endophthalmitis

Infectious endophthalmitis is a rare but potentially blinding ocular condition which can occur in the setting of intraocular surgery, trauma, or dissemination from endogenous non-ocular sources in the body. In the landmark Endophthalmitis Vitrectomy Study, gram-negative bacteria were isolated in only 4% of total and 5.9% of culture-proven postoperative endophthalmitis cases.[1] However, two similar studies from Southern India have revealed a higher proportion of gram-negative isolates in culture-proven endophthalmitis, one reporting 41.7% of postoperative cases,[2] while the other showing 30.1% of overall cases.[3] Pseudomonas was the predominant gram-negative organism in both the studies accounting for 25.9% and 13% of all isolates, respectively, being the single most frequent organism in the first study.[2,3] Endophthalmitis caused by Pseudomonas is characterized by a rapid fulminant course and poor visual and anatomical outcome even when the organism is sensitive to intraocular antibiotics.[4] Drug resistance to Pseudomonas further complicates the management and worsens the prognosis.[3] One of the studies mentioned above, in 2011, revealed Pseudomonas to be the culprit in 72.73% of multidrug-resistant (MDR)

bacterial endophthalmitis cases.[3] This prompted us to look back into all pseudomonas endophthalmitis cases in our institute and to compare the antibiotic sensitivity trends and the presence of MDR in two different time frames.

Methods

The study is a retrospective comparative consecutive series of all culture-proven Pseudomonas endophthalmitis cases treated from June 2004 to May 2016. The study aims to assess the antibiotic sensitivity trends of Pseudomonas. The study period is divided into two precisely similar 6-year time frames to assess temporal variation of antibiotic sensitivity avoiding as much bias as possible. The study protocol was approved by the institutional ethics committee and the study adhered to the tenets of the Declaration of Helsinki.

The microbiology department rigorously maintains a register for all ocular samples received for staining and culture. The "clinical diagnosis" column of the same was checked retrospectively for the term "endophthalmitis" during the timespan mentioned above. Further information was procured from the microbiology register itself. Any clinical details, like the cause of endophthalmitis, if not available were checked

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in the medical records department with the specific medical records number of the patient.

A case of endophthalmitis was diagnosed clinically as per the institute protocol. A detailed history of pain, vision loss, redness, duration of symptoms and their timeline from any ocular insult, any recent intraocular surgery, any trauma, any ocular surface infection, or presence of any non-ocular infective source in the body was noted. Meticulous documentation of visual acuity, anterior segment findings of any corneal edema or ulcer, anterior chamber cells, flare, hypopyon, fibrin membrane, and lens status were done by slit-lamp biomicroscopy. The grade of media clarity on indirect ophthalmoscopy and findings of vitritis, vitreous exudates, or any retinal exudates were recorded at presentation and on daily follow-ups. Confirmation and monitoring of vitreous membranes and exudates were done on consecutive ultrasound B-scans.

In all cases, vitreous specimen (0.2 ml) was procured on the day of presentation, and empirical treatment with intravitreal vancomycin (1mg in 0.1 ml) and ceftazidime (2.25 mg in 0.1 ml) was started along with topical and oral medications, as per our institutional protocol. The aspirated fluid was immediately plated on 5% sheep blood agar, chocolate agar, thioglycolate broth, brain heart infusion broth, and potato dextrose agar (HiMedia, India) as per the standard guidelines in the microbiology laboratory of our institute. Smears were also prepared for Gram's staining (HiMedia Gram Stain Kit K001-1 KT) and 10% potassium hydroxide wet mount. All inoculated media were incubated at 37°C except potato dextrose agar, which was done at 25°C for the growth of fungi. Blood agar plates were incubated both in aerobic and anaerobic conditions, and chocolate agar was incubated with 5% carbon dioxide. Antibiotic sensitivity was tested on Muller Hinton agar by Kirby–Bauer disc diffusion technique as per the latest Clinical and Laboratory Standards Institute guidelines. Pseudomonas was diagnosed based on the following criteria:[5]
1) Gram-negative bacilli with gliding motility
2) Growth of more than two lytic colonies on at least two media or confluent growth at the site of inoculation on one solid medium
3) Lactose and glucose nonfermenters on triple sugar iron agar
4) Positive oxidase, catalase and citrate reaction, and negative indole reaction.

All clinically diagnosed endophthalmitis cases at our hospital over 12 years were reviewed for positive culture growth from the vitreous specimen. Culture positive cases were screened for pseudomonas growth, and sensitivity data to commonly used antibiotic agents were recorded. The sensitivity outcomes of individual cases in antibiogram were mostly "sensitive" or "resistant" with few "intermediate sensitive" results. Intermediate sensitive means that the organism is inhibited *in vitro*, but the therapeutic effect is uncertain, indicating the need for heavier doses of antibiotics in clinical

use. To refute our hypothesis that the sensitivity pattern has changed over the last decade and to avoid any bias toward resistance, we included all "Intermediate sensitive" results under the "sensitive" category.

Statistical analysis

For analysis, cases were divided into two groups: group 1 included cases presenting in initial 6 years (June 2004—May 2010) and group 2 had cases presenting in final 6 years (June 2010–May 2016). Each of the two groups was further divided into three subgroups, namely subgroup 1A, 2A for postoperative, subgroup 1B, 2B for traumatic and subgroup 1C, 2C for endogenous cases. Sensitivity to individual antibiotics and percentage of MDR strains in all six subgroups were analyzed. MDR was defined as resistance to two or more different groups of typically susceptible antibiotics.[3] Other classes of antibiotics to which Pseudomonas has intrinsic resistance like vancomycin, second-generation penicillin, macrolides, tetracycline, narrow-spectrum cephalosporins were not considered while calculating MDR. Antibiotic sensitivity and percentage of MDR strains were analyzed and compared with Fisher's exact test.

Results

Of a total of 816 patients of clinically diagnosed endophthalmitis treated at our institute between June 2004 and May 2016, 389 cases showed positive growth on culture. Pseudomonas accounted for 74 of 389 culture positive isolates (19%). Of 74 cases of Pseudomonas endophthalmitis, 42 (56.8%) were in initial 6 years and 32 (43.2%) in the final 6 years. 63.5% (*n* = 47) were postoperative, 25.7% (*n* = 19) were traumatic, and 10.8% (*n* = 8) were endogenous cases [Table 1]. Sensitivity to ciprofloxacin, ofloxacin, gatifloxacin, and moxifloxacin was 85.7%, 82.9%, 76.5%, 76.9% up to 2010 which dropped to 75%, 59.4%, 68.8%, 56.3%, respectively, from 2010, being statistically significant only for ofloxacin (P = 0.0349). Susceptibility to amikacin, gentamicin, and tobramycin changed from 83.3%, 43.9%, 47.6% to 71.9%, 61.3%, 61.3%, respectively; none were statistically significant. Ceftazidime sensitivity values dropped significantly from 88.1% to 56.3% (P = 0.0028) from initial to final 6 years. Overall sensitivity to chloramphenicol improved from 11.1% to 58.6% (P = 0.0003). Sensitivity data for antibiotics only tested after 2010 were 96.3%, 71%, 65.5% for polymyxin, piperacillin-tazobactam, levofloxacin and 40%, 25% for tetracycline and cefuroxime. Comparison between two postoperative endophthalmitis subgroups threw more light on the dramatic shift in sensitivity trends as shown in Fig. 1. On the other hand, for traumatic endophthalmitis cases in initial (Gr 1B) versus final 6 years (Gr 1B), sensitivity of chloramphenicol (12.5% vs. 55.6%), all fluoroquinolones (ciprofloxacin 55.6% vs. 80%, ofloxacin 44.4% vs. 80%, moxifloxacin 57.1% vs. 80%, Gatifloxacin 50% vs 80%) and aminoglycosides (amikacin 66.7% vs. 90%, gentamicin 44.4% vs. 77.8%, tobramycin 44.4% vs. 80%) improved, whereas it remained almost same for ceftazidime (77.8% vs. 80%).

Table 1: Distribution of pseudomonas endophthalmitis cases according to cause and time-frame of presentation

Total - 74 (100%)	Group 1-42 (56.8%)	Group 2-32 (43.2%)
Postoperative cases - 47 (63.5%)	Subgroup 1A -29 (39.2%)	Subgroup 2A -18 (24.3%)
Traumatic cases -19 (25.7%)	Subgroup 1B -9 (12.2%)	Subgroup 2B -10 (13.5%)
Endogenous cases -8 (10.8%)	Subgroup 1C -4 (5.4%)	Subgroup 2C -4 (5.4%)

MDR was noted in 20 out of 74 cases (27%) in overall 12 years. MDR was only 16.7% (7 of 42) in the first 6 years compared to 40.6% (13 of 32) in the final 6 years, the *P* value is 0.0337. MDR was noted mostly in traumatic etiology (4 of 9 cases) in group 1, whereas it was primarily observed in postoperative cases (9 out of 18) in group 2. Percentage of MDR in postoperative cases rose alarmingly from 10.3% before 2010 to 50% after 2010 (*P* = 0.0048).

Discussion

In the Endophthalmitis Vitrectomy Study conducted from 1990 to 1994, 17 of 420 clinically diagnosed postoperative endophthalmitis cases were caused by gram-negative bacteria accounting for 4% of overall and 5.9% of culture-positive cases.[1] 89% of these gram-negative bacteria were susceptible to both amikacin and ceftazidime.[1] Eifrig *et al.* found only 2 MDR isolates of 28 pseudomonas cases (7%) from 1987 to 2001.[4] The same institute had only 12 cases in the next 10 years with all sensitive strains and minimum inhibitory concentration 90 of antibiotics remaining same.[6] Chen from Taiwan reported 100% sensitivity to ceftazidime and excellent sensitivity to other drugs.[7] Similar case series from Brazil[8] and Tehran[9] noted no drug resistance to Pseudomonas isolates. A notable exception was observed in 12 reported cases from Greece where all the cases were MDR with susceptibility only to carbapenems and colistin prompting intraocular colistin use.[10] Table 2 elucidates the antibiotic sensitivity patterns and percentage of MDR from studies outside India.

Figure 1: Comparison of antibiotic sensitivity between two postoperative endophthalmitis subgroups (Group 1A vs 2A). CH – Chloramphenicol, CA – Ceftazidime, CF – Ciprofloxacin, OF – Ofloxacin, MO – Moxifloxacin, GF – Gatifloxacin, AK – Amikacin, G – Gentamicin, TB – Tobramycin, CE – Cephotaxime

However, studies from India depict a different scenario. Pseudomonas accounted for a high proportion of culture-positive isolates in four studies from Southern India ranging from 13.1% to 25.9%.[2,3,11,12] Antibiotic sensitivity trends show reduced sensitivity to ceftazidime, ciprofloxacin, and amikacin in these studies [Table 3] compared to those done outside India [Table 2]. Pathengay *et al.*[3] and Jindal *et al.*[12] from the same institute but in different time frames reported a similar proportion of MDR cases but a dramatic drop in sensitivity to ceftazidime and amikacin in MDR cases. Our study confirms this trend of rising resistance to ceftazidime and amikacin in postoperative cases [Fig. 1]. As regards to traumatic cases in our study, flouroquinolones and amikacin showed better sensitivity in recent years. Since the number of cases were only 9 in group 1B and 10 in group 2B, this reverse trend if at all may be an incidental finding or related to pseudomonas intraspecies variation due to different source of infection in traumatic cases compared to hospital setting.

Pinna *et al.* found their MDR cases to be resistant to all antibiotics tested except polymyxin B.[13] Samant *et al.* noted resistant to all antibiotics except colistin in all 8 cases.[14] Our MDR strains were 100% resistant to chloramphenicol, 90% to gentamicin, 75% to ceftazidime, 85% to ofloxacin, 80% to gatifloxacin, 70% to ciprofloxacin and amikacin, 89.5% to tobramycin, 88.9% to moxifloxacin, 75% to levofloxacin, 90% to tetracycline, 65% to cefotaxime, 69.2% to piperacillin-tazobactam (PIP/TZ), and only 9.1% to polymyxin. Four of 7 MDR cases up to 2010 were resistant to all antibiotics but sensitivity to PIP/TZ and polymyxin was not tested. In the next 6 years, 4 of 13 MDR cases were resistant to all antibiotics including PIP/TZ except polymyxin B. The latest case was resistant even to polymyxin but fortunately sensitive to amikacin and tobramycin. Interestingly, sensitivity to chloramphenicol showed massive improvement in recent 6 years, probably attributed to minimal systemic and ocular use.

Analysis of all the studies shows significant temporal and geographic variations in antibiotic sensitivity of Pseudomonas. Studies from India particularly in recent times show increased resistance of Pseudomonas to aminoglycosides, fluoroquinolones, and cephalosporins. Our study, which for the first time compared the sensitivity of Pseudomonas in two different time frames, not only validates the upward trend in resistance to fluoroquinolones, amikacin, and ceftazidime but also reports a rise in MDR (1 in 4 cases) in Pseudomonas species. Pseudomonas is notorious for antibiotic resistance. It can acquire resistance through multiple mechanisms, notable
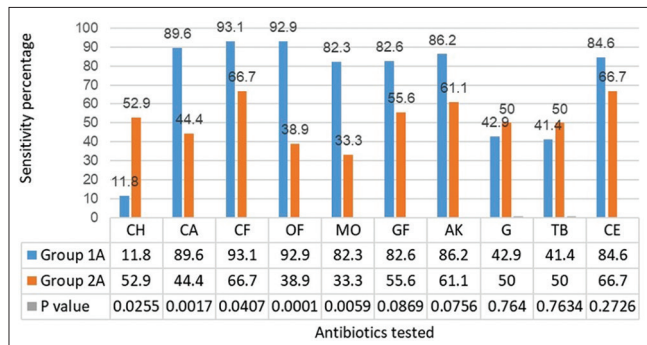
Table 2: Antibiotic sensitivity patterns and percentage of MDR from studies outside India

First author, place, period of study	No of cases	Sensitivity percentage of Antibiotics Tested										MDR %
		CA	CF	LF	AK	G	TB	CE	IPM	PIP/TZ	CST	
Eifrig,[4] US, 1987-2001	28	92.8%	92.8%	NA	92.8%	92.8%	92.8%	NA	NA	NA	NA	2 7%
Chen,[7] Taiwan, 1997-07	72 (16%)	100%	93%	NA	94%	86%	NA	NA	99%	96%	NA	0%
Sridhar,[6] US, 2002-12	12	100%	92%	100%	92%	NA	93%	NA	90%	NA	NA	0%
Guerra,[8] Brazil, 2009	26	100%	NA	NA	100%	100%	NA	100%	100%	100%	NA	0%
Falavarjani,[9] Tehran, 2005-15	20	83.4%	100%	NA	88.3%	76.5%	76.5%	38.5%	100%	NA	NA	0%
Maltezou,[10] Greece, 2010	12	NA	0%	0%	0%	0%	0%	NA	100%	0%	100%	12 100%

CA – Ceftazidime; CF – Ciprofloxacin; LF – Levofloxacin; AK – Amikacin; G – Gentamicin; TB – Tobramycin; CE – Cephotaxime; IPM – Imipenem; PIP/TZ – Piperacillin/Tazobactam; CST – Colistin; MDR – multidrug resistance; NA – not available

Table 3: Antibiotic sensitivity patterns and percentage of MDR from studies in India

First author, place, period of study	No of cases	Sensitivity percentage of Antibiotics Tested								MDR%
		CA	CF	OF	GF	MO	AK	G	Others	
Kunimoto,[11] Southern India, 1991-97	25 (19.8%)	68.8%	88%	NA	NA	NA	85.7%	52%	NA	
Anand,[2] Southern India, 1995-98	44 (25.9%)	62.5%	73.2%	NA	NA	NA	68.1%	55%	CE 65%	
Pathengay*,[3] Southern India, 2000-07	106 (13.1%)	54.5% (12/22)	52.2% (12/23)	18% (2/11)	20% (2/10)	NA	45.8% (11/24)	0% (0/22)	CH 0% (0/18)	24 22.6%
Jindal*,[12] Southern India, 2005-2010	38 (23.2%)	0%	50%	50%	50%	NA	0%	0%	IPM 37.5% PIP/TZ 0%	8 21%
Pinna,[13] Southern India, 2008	20	NA	20%	10%	15%	10%	0%	5%	PLB 100%	20 100%
Samant,[14] Western India, 2013	8	Resistant to all antibiotics except colistin								8 100%
Our study, Southern India, 2004-2010	42	88.1%	85.7%	82.9%	76.5%	76.9%	83.3%	43.9%	TB 47.6% CE 80.6%	7 16.7%
Our study, Southern India, 2010-2016	32	56.3%	75%	59.4%	68.8%	56.3%	71.9%	61.3%	TB 61.3% CE 71.9%	13 40.6%

*Sensitivity profile of MDR only. CA – Ceftazidime; CF – Ciprofloxacin; OF – Ofloxacin; GF- Gatifloxacin; MO- Moxifloxacin; AK – Amikacin; G – Gentamicin; CE – Cephotaxime; CH – Chloramphenicol; TB – Tobramycin; IPM – Imipenem; PIP/TZ – Piperacillin/Tazobactam; PLB - Polymyxin; MDR – multidrug resistance; NA – not available

being active drug efflux pumps, aminoglycoside modifying enzymes, AmpC β-lactamase enzymes, mutation of a 30S ribosomal subunit, methylation of the aminoglycoside-binding site, and point mutations in gyrA/gyrB genes.[15] Injudicious use, improper dosage, and lack of compliance to the full course of antibiotics can contribute to drug resistance. Fluoroquinolones are rampantly used in ophthalmology, as well as in all other fields of medicine to treat a wide variety of diseases. Apart from treating ophthalmic infections, they are routinely used for prophylaxis before surgeries or intravitreal injections and in the postoperative regimen. Widespread and inappropriate use of antibiotics along with the cross-transfer of MDR among gram-negative organisms is the probable cause of the emergence of MDR. Critical analysis of indications for antibiotic use and selective reservation of certain drugs for severe infections is the need of the hour. The importance of strict vigilance of antibiotic usage and area-wise periodic review of the microbiological profile with antibiotic sensitivity cannot be overstated. This data raises a question mark on the usage of ceftazidime or amikacin as the first choice for empirical gram-negative coverage in geographical areas of India with a high percentage of resistant Pseudomonas cases since irreversible ocular damage occurs before sensitivity reports are available. Role of other antibiotics as empirical therapy or as reserve drug needs to be considered. In our study, polymyxin B (same group as colistin) and PIP/TZ had the best sensitivity profiles after 2010. In one study, 50% of the isolates resistant to both amikacin and ceftazidime were sensitive to imipenem,[12] but imipenem has not been used intravitreally as yet. Colistin has specific antimicrobial activity against gram-negative bacteria especially pseudomonas and its intravitreal use was reported to be safe.[14]

The study is a retrospective case series. The spectrum of antibiotics tested for gram-negative bacteria in our microbiology lab follows a set protocol where sensitivity to colistin and carbapenems are not done. Moreover, antibiotic susceptibility was tested by disc diffusion method and not confirmed by MIC. Ours being a tertiary center treating many complicated and referral cases, the results might not be the accurate reflection of susceptibility profile of Pseudomonas in general population of South India. This study did not focus on how MDR adversely affects the clinical outcome but will surely be assessed in a forthcoming study.

Conclusion

The current study shows the resistance of Pseudomonas to fluoroquinolones, amikacin, and cephalosporins is on the rise particularly in post-surgical cases, including common intravitreal drug ceftazidime. Sensitivity to other aminoglycosides has improved along with very promising results for polymyxin and piperacillin-tazobactam. MDR should be countered by appropriate use of broad-spectrum antibiotics, improving compliance to full treatment duration, judiciously prescribing available drugs and using new alternatives drugs.

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