



Prevalence and Molecular Characterization of Metallo β -Lactamase Producing Gram-Negative Pathogens Causing Eye Infections

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Purpose: Metallo β -lactamases (M β L) production is a worldwide problem, particularly in gram-negative bacteria. As scanty data is available on the prevalence of MBL, the present study is being undertaken to determine the prevalence, antibacterial sensitivity patterns, and molecular characterization of M β L associated resistant genes in gram-negative bacteria isolated from ocular infections.

Material and Methods: At a tertiary eye care center in south India, 359 gram-negative pathogens, 200 isolates from eye infections, and 159 isolates from normal flora of the eye were studied. A gold standard microbiology method was used to identify the isolates. An antibiotic double disc synergy test and a combination disc test were used to detect M β L production. Multiplex PCR was used to investigate the molecular characteristics of the M β L encoding genes *bla*_{VIM}, *bla*_{IMP}, and *bla*_{NDM}.

Results: Of the 359 gram-negative bacterial pathogens, *Pseudomonas aeruginosa* 108 (30.1%) and *Enterobacter agglomerans* 46 (12.8%) were commonly isolated. High prevalence of *P. aeruginosa* 81% (17 strains) was detected as an M β L producer and it shows 100% resistance to 2nd and 3rd generation cephalosporins and meropenem. Multiplex PCR detected only the *bla*_{VIM} gene in 56 (28%) of various eye infections and 27 (17%) of normal flora of the gram-negative bacteria (GNB). The *bla*_{VIM} gene is detected predominantly in 51.8% of keratitis and 21.4% of postoperative endophthalmitis. High prevalence of the gene was detected in *P. aeruginosa* 42.9% (24 of 56) and *Alcaligenes denitrificans* 10.7% (6 of 56) from eye infections. Whereas, in the control group, *P. aeruginosa* and *E. coli* each had 14.8% (4 of 27) that were shown positive.

Conclusion: The emerging M β LS mediated resistance among *P. aeruginosa* is a challenging task for ophthalmologists, especially in patients with endophthalmitis and bacterial keratitis. This local knowledge will aid in advising appropriate antibiotic use and avoiding unnecessary antibiotic prescriptions, which are highly warranted.

Keywords: Metallo β -lactamases, *Enterobacteriaceae*, non-*Enterobacteriaceae*, normal flora, gram-negative bacilli

INTRODUCTION

The eye is a unique organ with physical barriers that keep out infectious agents, yet due to the eye's surface invariability, it is susceptible to a vast variety of microbial infections (1). Microorganisms can permeate and damage the internal parts of the eye, which often results in irreversible loss of vision (2). Bacterial infections of the eye are most commonly caused by external sources such as penetrating trauma or by microorganisms conquering the intraocular via the bloodstream (3). Changes in normal flora can also cause external and internal eye infections (1, 4, 5). *Pseudomonas aeruginosa*, *Enterobacter species*, *Klebsiella pneumoniae*, and *Alcaligenes denitrificans* are the most common gram-negative bacteria that cause eye infections (6). Due to their stability against the majority of β -lactamases and high permeation rate through bacterial outer membranes, the β -lactam groups of antibiotics, such as carbapenem drugs, are potential agents in treating multidrug resistant gram-negative bacterial infections (7). However, the efficacy of these drugs is increasingly being limited because of the emergence of carbapenem-resistant pathogens world-wide, especially in Southeast Asia and Europe (8, 9).

Understanding antibacterial resistance is crucial for selecting appropriate antibiotics to control infection, thus preventing vision-threatening complications. Overuse of antibiotics for systemic infections and topical antibiotics for eye infections are both factors that contribute to the development of antibiotic resistance among ocular isolates (5). Routine antibacterial susceptibility disc diffusion testing, as well as molecular approaches for drug resistance detection, are highly recommended for understanding the incidence of antibiotic resistance to extended-spectrum antibiotics (6). The spread of resistance to β -lactam group antibiotics occurs through β -lactamase enzyme production by gram-negative bacteria and is an emerging problem. β -lactamase are divided into four groups, with categories A, C, and D having serine at their active site, and Metallo- β -lactamase (M β L) belonging to the Amber class B (10, 11). Most other β -lactam antibiotics, with the exemption of monobactam can be hydrolysed by the M β L generating bacterium, including penicillin, cephalosporin, and carbapenem (10, 11). Bacterial isolates that produce M β LS are also associated with increased morbidity and mortality (12). Mobile genetic components such as transposons, plasmids, and integrons, which have the ability to move both within and between species, contain M β L gene (10, 11).

The prevalence of different types of acquired M β LS genes today can be ascertained rapidly (13). The different forms of M β L

genes have been discovered in gram-negative bacteria including (i) Verona integron- coded M β L (VIM), (ii) imipenemase (IMP), Seoul imipenemase (SIM), (iii) Germany imipenemase (GIM), (iv) Spaulo M β L (SPM), (v) New delhi M β L (NDM). VIM and IMP are the most common inherited M β L genotypes in India (14, 15). The incidence of M β LS positive isolates in hospital settings is not only a therapeutic challenge but also a major problem in infection control management. Early detection of the prevalence and types of M β LS is very important, with benefits including timely implementation of strict infection control measures and treatment with different antimicrobials. M β L genes have spread from *Pseudomonas aeruginosa* to the members of the *Enterobacteriaceae* family in recent years (16). In India, the M β LS have been found in a variety of systemic infections, but only one center has reported on ocular infections (17). The purpose of this study was to determine the prevalence and antibacterial susceptibility profile of M β L producing gram-negative bacteria isolated from various eye infections and normal flora of the eye (preoperative conjunctival flora, i.e., control group).

MATERIALS AND METHODS

Bacterial Isolates and Research Subjects

This study only includes gram-negative bacteria isolated from blepharitis, conjunctivitis, contact lens associated keratitis, bacterial keratitis, dacryocystitis, canaliculitis, and endophthalmitis, as well as samples from patients with penetrating and therapeutic keratoplasty surgeries, post suture infection, and normal flora of the eye (preoperative conjunctival swabs collected from patients who underwent cataract surgeries, i.e., control group) between January 2014 and December 2016, who were treated at a tertiary eye care referral center in South India. The study was approved by the institutional review board and follows the tenets of the Declaration of Helsinki. After detailed slit-lamp biomicroscopic examination, specimens were collected by ophthalmologists for direct microscopic observation and cultures. The samples were inoculated onto the 5% sheep blood agar (M450 -Tryptose blood Agar base), chocolate agar, potato dextrose agar (GM096), thioglycollate broth (M009), and brain heart infusion broth (BHI) (M210) separately (Hi-Media Laboratories Pvt. Ltd, Mumbai, India). After inoculation of specimen onto the culture media were incubated at 37°C for the growth of bacteria, chocolate agar were incubated in 3–5% CO₂ incubator (Thermo Scientific, Model: 3111, HEPA Class 100, USA) at 37°C, Potato dextrose agar were incubated at BOD incubator (Techlab, SZ135, India) at 25–27°C for the growth of fungi. After the growth in the media, the pure culture of

the organism was identified up to species level using standard biochemical tests. For this study, only gram-negative bacteria were included for further analysis.

Antibacterial Susceptibility Testing

The Kirby-Bauer disc diffusion method was used to test antibacterial susceptibility *in vitro* for each pure bacterial isolate, and the results were interpreted using the Clinical and Laboratory Standards Institute's serum standards (CLSI) (2012) (18). The antibacterial agents (BIO-RAD Laboratories, France) used were amikacin (30 µg), tobramycin (10 µg), gentamicin (10 µg), cefazolin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefpodoxime (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), gatifloxacin (5 µg), moxifloxacin (5 µg), chloramphenicol (30 µg), imipenem (10 µg), meropenem (10 µg), aztreonam (10 µg), ceftazidime (30 µg), ceftiofur (30 µg), piperacillin (10 µg), piperacillin/tazobactam (100/10 µg), ticarcillin (75 µg) ticarcillin/clavulanic acid (75/10 µg), ceftriaxone (30 µg), and cefotetan (30 µg). As a reference and quality control, standard American Type Culture Collection (ATCC) bacterial isolates were used (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922).

Phenotypic Detection of MβL Activity Combined Disc Test

The combined disc test was carried out as per the method advocated by Yong et al. (19). The isolate was adjusted to the 0.5 McFarland standard by Muller-Hinton (MH) broth and swabbed on the MH agar plates. Imipenem (10 microgram) and meropenem discs (10 microgram) were placed on the inoculated agar plate, and 10 µL of 0.5 M EDTA solution was impregnated onto each antibiotic disc. The plates were incubated overnight at 37°C. After incubation, the zone of inhibition was measured and compared. Imipenem and imipenem integrated with EDTA disc or meropenem and meropenem integrated with EDTA discs showed a zone of inhibition >7 mm which was considered to be positive MβLs production of the isolate.

Double Disc Synergy Assay

This assay was conducted based on Arakawa et al. (20) and Lee et al. (21) with modifications. The MH agar plate was inoculated with the isolate of 0.5 McFarland standard. A 10 microgram imipenem disc or a 10 microgram meropenem disc was put down on the agar plate. A sterile disc (6 mm) was impregnated with 10 µL of 0.5 M EDTA solution. These plates were incubated overnight at 37°C. After incubation, the plates were observed for synergistic activity.

Molecular Characterization of MβL Genes Preparation of Template DNA

A single colony was inoculated into 5 mL of Luria-Bertani broth (Himedia, Mumbai) and incubated for 20 h at 37°C. Cells were harvested from a 1.5 mL culture by centrifugation at 12,000 rpm for 5 min. According to the manufacturer's instructions, DNA was extracted using the QIAamp® DNA micro kit (51304). (QIAGEN, Hilden, Germany). DNA was extracted from the test isolates along with the *bla*_{VIM} gene carrying

P. aeruginosa 1138 (GenBank accession number KC505236) (positive control for *bla*_{VIM-2} gene), *bla*_{IMP} gene carrying *P. aeruginosa* R-61 (GenBank accession number KF570107) (positive control for *bla*_{IMP-1} gene) and *bla*_{NDM} gene carrying *Klebsiella pneumoniae* BC5500 (Genbank accession number KF570106) (positive control for *bla*_{NDM-1} gene). As a negative control, a non-MβL producing *P. aeruginosa* ATCC 27853 was used.

Gene-Specific Oligonucleotide Primer Pairs

The gene specific primers, *bla*_{VIM}-F- 5'-GATGGTGTGTTGGTTCGCATA-3' and *bla*_{VIM}-R- 5'-CGAATGCGCAGCACCAG-3' which amplified a 390 bp amplicon, *bla*_{IMP}- F-5'- TTAGACTCCATTTACDG-3' and *bla*_{IMP}-R-5'-GATYGAGAATTAAGCCACYCT-3' which amplified a 139bp amplicon as described by Dallenne et al. (22) and *bla*_{NDM} -F-5'-CCAGCTTGCCCCGCAAGAGG-3' and *bla*_{NDM}-R-5'ATCGGGGGCGGAATGGCTCA- 3' which amplified a 350 bp amplicon were selected for study (22).

Multiplex Polymerase Chain Reaction for *Blavim*, *Blaimp*, and *Blaindm*-Gene Identification

Multiplex PCRs were performed in the Eppendorf Mastercycler ProS (Hamburg, Germany) with a final volume of 25 µL in 0.2 mL thin-walled PCR tubes. Each reaction contained 2.5 µL of 10X PCR buffer (10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl₂), 0.5 µL dNTP's (10 µM), 1 µL (each) primers (10 pmol/mL) with 0.2 µL (3 U/µL) *Taq* DNA polymerase (Bangalore Genei, Bangalore, India) was added to the reaction mixture. The multiplex PCR cycling conditions for amplification were as follows: The initial denaturation was at 94°C for 5 min and 30 cycles, 40 s at 94°C, 40 s at 55°C, and 1 min at 72°C, followed by a final extension of 7 min at 72°C for *bla*_{VIM}, *bla*_{IMP}, and *bla*_{NDM}-genes. After the amplification reaction, PCR products were analyzed by electrophoresis on 1.5% agarose gels.

TABLE 1 | List of specimens study included based on the diagnosis (*n* = 355).

Diagnosis	Specimen	Total (<i>n</i> = 355)
Keratitis (<i>n</i> = 121)	Corneal scraping	52
	Contact lens solution	36
	Corneal button	30
	Bandage contact lens	3
	Conjunctivitis	Conjunctival swab
Endophthalmitis (<i>n</i> = 26)*	Vitreous fluid	25
	IOL	1
Dacryocystitis	Pus	12
Suture infection (<i>n</i> = 5)	Suture material	2
	Foreign body	3
Canaliculitis	Lid abscess	2
Blepharitis	Lid swab	1
Pre-operative conjunctival flora (Control group)	Conjunctival swab	159

* Post-operative (*n* = 21), Trauma (*n* = 2), Post IVTA (*n* = 1) and Due to keratitis (*n* = 1). Bold values show the total in numbers for respective category.

RESULTS

Bacterial Isolates and Study Subjects

A total of 359 gram-negative bacterial isolates were isolated from 355 patients during the study period. Of which 159 (44%) isolates were from the control group (preoperative conjunctival flora from patients with no infection who underwent cataract surgery), and the remaining 200 (56%) isolates were from various ocular infections. The major disease samples received during the study duration are corneal scrapings from keratitis patients (52 of 200; 26%), followed by contact-lens associated keratitis (36 of 200; 18%), conjunctivitis (29 of 200; 14.5%), and endophthalmitis (26 of 200; 13%) (Table 1). The highest number of gram-negative bacterial species isolated from the control group was *K. pneumoniae* (23 of 159; 14.5%), followed by *P. aeruginosa* (22 of 159; 13.8%), and *E. agglomerans* (21 of 159; 13.2%). In the infection group, *P. aeruginosa* (86 of 200; 43%) was the predominant isolate, followed by *E. agglomerans* (25 of 200; 12.5%), *K. pneumoniae* (16 of 200; 8%) and *E. coli* (11 of 200; 5.5%) (Table 2).

Antibacterial Susceptibility Test

The antibiotic resistance profile of the 359 gram-negative bacterial isolates is presented in Table 3. *P. aeruginosa* isolates

from both infection and control groups showed 100% resistance to cefoxitin, cefotetan, and cefpodoxime. In the carbapenem group, 29 and 23% were resistant to meropenem in both infection and control groups, respectively. In the fluoroquinolone group tested antibacterial which showed the resistance range from 30 to 34% in infection group and 14 to 18% in control group. *Enterobacteriaceae* isolates from infection showed 73% resistance to cefazolin, cefpodoxime, and piperacillin each at 67%. Whereas, in the control group, 72% of isolates were resistance to piperacillin and 62% to ticarcillin. In the carbapenem group, imipenem showed 30% resistance in both infection and control group *Enterobacteriaceae* isolates. The fluoroquinolone group tested antibacterial, which showed a resistance range of 18 to 33% in the infection group and 17 to 25% in the control group (Figure 1).

Phenotypic Detection of MβLs Activity

The production of MβLs was tested by a combined disc test and a double disc synergy test. Only 10.5% (21 of 200) and 0.6% (1 of 159) of the ocular infection and normal flora organisms exhibited MβL activity. Of which, 81% (17 of 21) were *P. aeruginosa*, 4.8% (1 of 21) were *Aeromonas hydrophila*, and *Acinetobacter lwoffii*, *Alcaligenes denitrificans*, and *Alcaligenes faecalis* each had 4.8% (1 of 21), respectively. Only one *A. hydrophila* isolate

TABLE 2 | Distribution of gram-negative isolates from various ocular infections and pre-operative conjunctival flora (Control group).

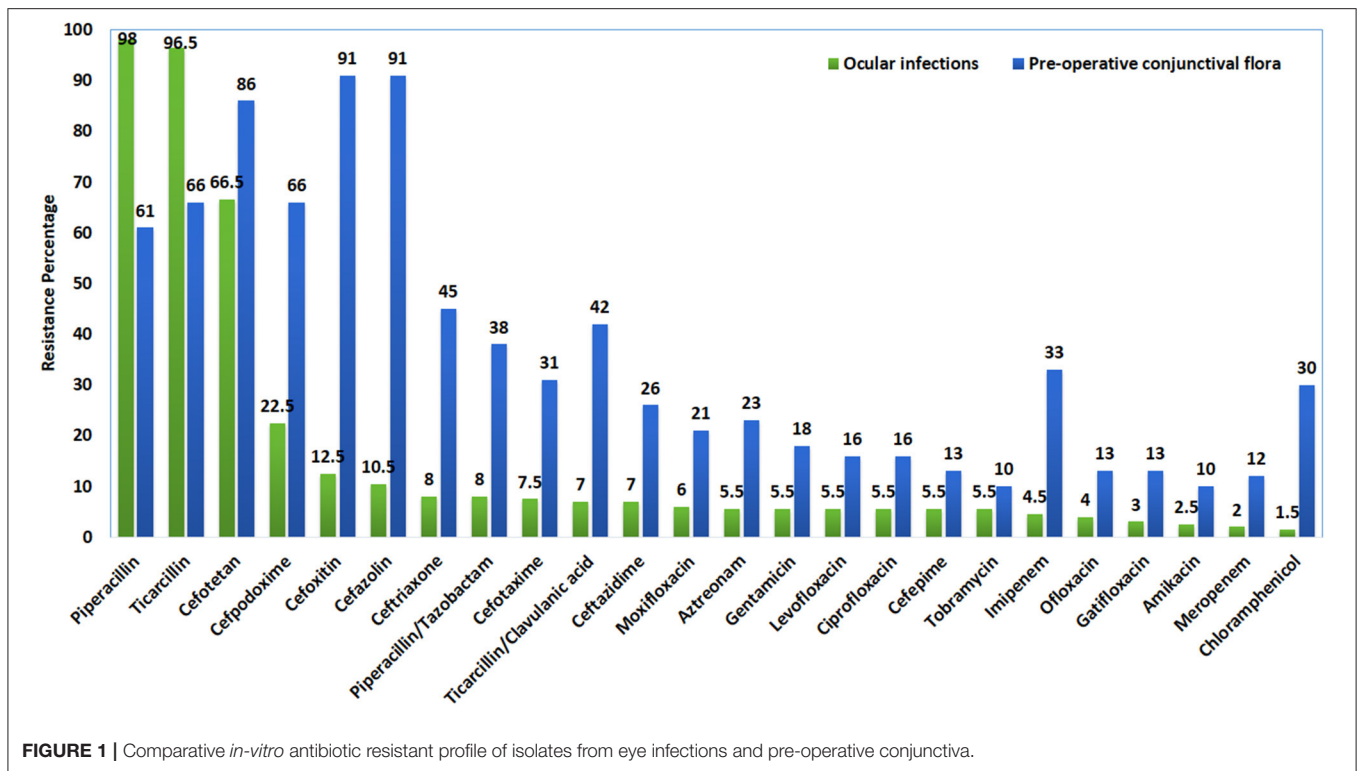
Name of the bacterial isolates	Ocular infections		Pre-operative conjunctival flora (Control group)		Total	
	n	%	n	%	n	%
Non-Enterobacteriaceae	127	63.5	59	37.1	186	51.8
<i>P. aeruginosa</i>	86	43	22	13.8	108	30.1
<i>P. alcaligenes</i>	16	8	3	1.9	19	5.3
<i>A. denitrificans</i>	10	5	6	3.8	16	4.5
<i>A. faecalis</i>	7	3.5	5	3.1	12	3.3
<i>A. lwoffii</i>	5	2.5	7	4.4	12	3.3
<i>A. hydrophila</i>	3	1.5	16	10.1	19	5.3
Enterobacteriaceae	73	36.5	100	62.9	173	48.2
<i>E. agglomerans</i>	25	12.5	21	13.2	46	12.8
<i>K. pneumoniae</i>	16	8	23	14.5	39	10.9
<i>E. coli</i>	11	5.5	13	8.2	24	6.7
<i>S. marcescens</i>	10	5	1	0.6	11	3.1
<i>E. aerogenes</i>	5	2.5	3	1.9	8	2.2
<i>C. freundii</i>	2	1	1	0.6	3	0.8
<i>K. oxytoca</i>	2	1	1	0.6	3	0.8
<i>M. morgonii</i>	1	0.5	6	3.8	7	1.9
<i>P. rettgeri</i>	1	0.5	1	0.6	2	0.6
<i>P. mirabilis</i>	0	0	11	6.9	11	3.1
<i>C. diversus</i>	0	0	10	6.3	10	2.8
<i>C. koseri</i>	0	0	6	3.8	6	1.7
<i>E. cloacae</i>	0	0	1	0.6	1	0.3
<i>P. vulgaris</i>	0	0	1	0.6	1	0.3
<i>V. furnissii</i>	0	0	1	0.6	1	0.3
Total in numbers (%)	200	100	159	100	359	100

Bold values show the total in numbers for respective category.

TABLE 3 | *In-vitro* antibiotic susceptibility profile of Non-*Enterobacteriaceae* vs. *Enterobacteriaceae* from infection vs. control group.

Name of the antibacterial agents	Non- <i>Enterobacteriaceae</i>								<i>Enterobacteriaceae</i>			
	Infection group				Control group*				Infection group		Control group*	
	<i>P. aeruginosa</i> (n = 86)	%	Other non- <i>Enterobacteriaceae</i> (n = 41)		<i>P. aeruginosa</i> (n = 22)	%	Other non- <i>Enterobacteriaceae</i> (n = 37)	%	n = 73	%	n = 100	%
Carbapenems												
Meropenem (10 mcg)	25	29	7	17	5	23	4	11	4	5	10	10
Imipenem (10 mcg)	9	10	10	24	0	0	22	59	22	30	30	30
Fluroquinolones												
Levofloxacin (5 mcg)	29	34	6	15	4	18	2	5	20	27	19	19
Moxifloxacin (5 mcg)	29	34	7	17	4	18	4	11	24	33	25	25
Ofloxacin (5 mcg)	28	33	5	12	4	18	0	0	13	18	17	17
Ciprofloxacin (5 mcg)	27	31	5	12	3	14	2	5	23	32	20	20
Gatifloxacin (5 mcg)	26	30	5	12	4	18	2	5	14	19	18	18
Cephalosporins												
2nd generation												
Cefoxitin (30 mcg)	86	100	39	95	22	100	35	95	30	41	22	22
Cefotetan (30 mcg)	86	100	35	85	22	100	18	49	12	16	10	10
3rd generation												
Cepfodoxime (10 mcg)	86	100	41	100	22	100	31	84	49	67	52	52
Ceftriaxone (30 mcg)	86	100	37	90	21	95	25	68	27	37	26	26
Cefotaxime (30 mcg)	57	66	21	51	10	45	13	35	31	42	26	26
Ceftazidime (30 mcg)	46	53	19	46	17	77	12	32	29	40	24	24

*Control group 96 Preoperative conjunctival flora.



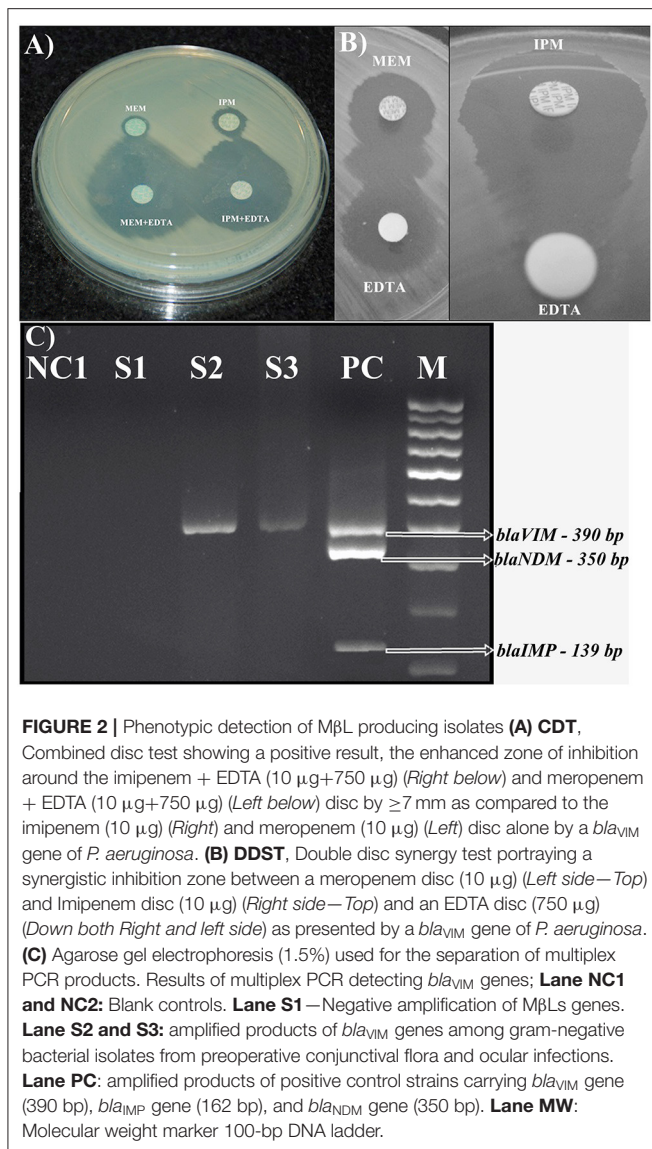
was positive for M β L producer from pre-operative conjunctiva. The highest number of M β Ls producers was observed in isolates from vitreous fluid specimens (36%), followed by corneal scraping (18%), contact lens solution (14%) and conjunctival swab specimens (9%) (**Supplementary Table 1** and **Figures 2A,B**).

Table 4 summarizes the results of the antibacterial susceptibility profile of M β L and non-M β L generating *P. aeruginosa* and Enterobacteriaceae. Out of the 108 (30%) *P. aeruginosa* isolates tested, 16% (17 of 108) were M β Ls producers, and 84% (91 of 108) were non-M β L producers. Among the 17 M β L producing *P. aeruginosa* from infection group which showed the susceptibility to antibacterials were in the following order: aztreonam (59%), imipenem (47%), and cefepime (41%), while the resistance percentage to other antibacterials were in the following order: cefazolin (100%), cefpodoxime (100%), cefotetan (100%), ticarcillin (100%) and ticarcillin/clavulanic acid (100%), meropenem (100%), and gentamicin (94%). Of the 69 non-M β L producing *P. aeruginosa*, the susceptibility to antibacterials was in the following order: imipenem (100%), amikacin (90%), tobramycin (87%), and meropenem (88%). Hundred percentage of resistance was recorded for 2nd generation cephalosporins (cefoxitin and cefotetan), cefpodoxime, and ceftriaxone, and 99% of resistance was recorded for ticarcillin and ticarcillin with clavulanic acid. *P. aeruginosa* isolates (non-M β L producers) in the control group displayed the same resistant patterns as the infection group.

Molecular Detection of M β L Genes

The Multiplex polymerase chain reaction assay detected 23% (83 of 359) of GNB isolates carrying only M β L encoding *bla*_{VIM} gene. There were 28% (56 of 200) *bla*_{VIM} gene-positive isolates from ocular infections and 17% (27 of 159) from pre-operative conjunctiva. The *bla*_{VIM} gene positive isolates were detected predominantly from 51.8% keratitis, 21.4% postoperative endophthalmitis, 17.9% conjunctivitis, and 34.1% from preoperative conjunctiva. Among the 56 isolates from ocular infections, 44 (78.6%) *bla*_{VIM} genes were detected in non-Enterobacteriaceae isolates and 12 (21.4%) among Enterobacteriaceae members. In the control group, 17 (63%) and 10 (37%), respectively, isolates were from the Enterobacteriaceae and non-Enterobacteriaceae families. A high prevalence of *bla*_{VIM} gene were detected from *P. aeruginosa* 42.9% (24 of 56) and *Alcaligenes denitrificans* 10.7% (6 of 56) in the ocular infections and in the control group, a high number of *P. aeruginosa* and *E. coli*, each 14.8% (4 of 27), were detected (**Supplementary Table 1** and **Figure 2C**).

Despite the presence of genes in 83 organisms, only 22 isolates exhibited phenotypic expression. There were 54 genotypically positive isolates among non-Enterobacteriaceae members and 29 genotypically positive isolates among Enterobacteriaceae members. Even though they have the genes, none of the Enterobacteriaceae members express the phenotype in both the control and infection groups. Interestingly, among non-Enterobacteriaceae members, 21 phenotypically positive isolates



belonged to the infection group, whereas only one isolate belonged to the control group (Figures 3A,B).

DISCUSSION

The ocular surface supports and maintains a small population of bacteria as normal flora with a combination of mechanical, anatomical, immunological, and microbiological factors (23). Previous studies reported the positive growth of microbes up to 83% in normal healthy conjunctiva (5), typically with coagulase-negative *Staphylococci* and occasionally with members of *Enterobacteriaceae* (24). Another study showed 4.3% of gram-negative bacterial growth was seen among patients who underwent cataract or glaucoma surgery. (5). The current study also confirmed the colonization of gram-negative pathogens in the preoperative conjunctiva with *Enterobacteriaceae* family members accounted for 62.9, and

37.1% were non-*Enterobacteriaceae* families. *P. aeruginosa* (52.9%) was the most common among gram-negative bacteria in causing both internal and external eye infections (3). The current study also identified *P. aeruginosa* (43%) as the predominant pathogen causing eye infections.

In the present analysis, the *in-vitro* susceptibility profile of preoperative conjunctival GNB flora (control group) showed high level susceptibility to amikacin, tobramycin, meropenem, and ofloxacin, and high level resistance to cefoxitin, cefotetan, ceftazidime, and ticarcillin. Studies from California have also shown a high level of resistance to ceftazidime and a low level of resistance to amikacin, whereas none of the gram-negative bacterial isolates were resistant to the fluoroquinolone antibiotic group (24). In spite of the defense by the tears along with the flicker action of the eyelids, the resident bacteria can become opportunistic to cause infection, which necessitates the importance of prophylactic antibiotic interventions before ocular surgery.

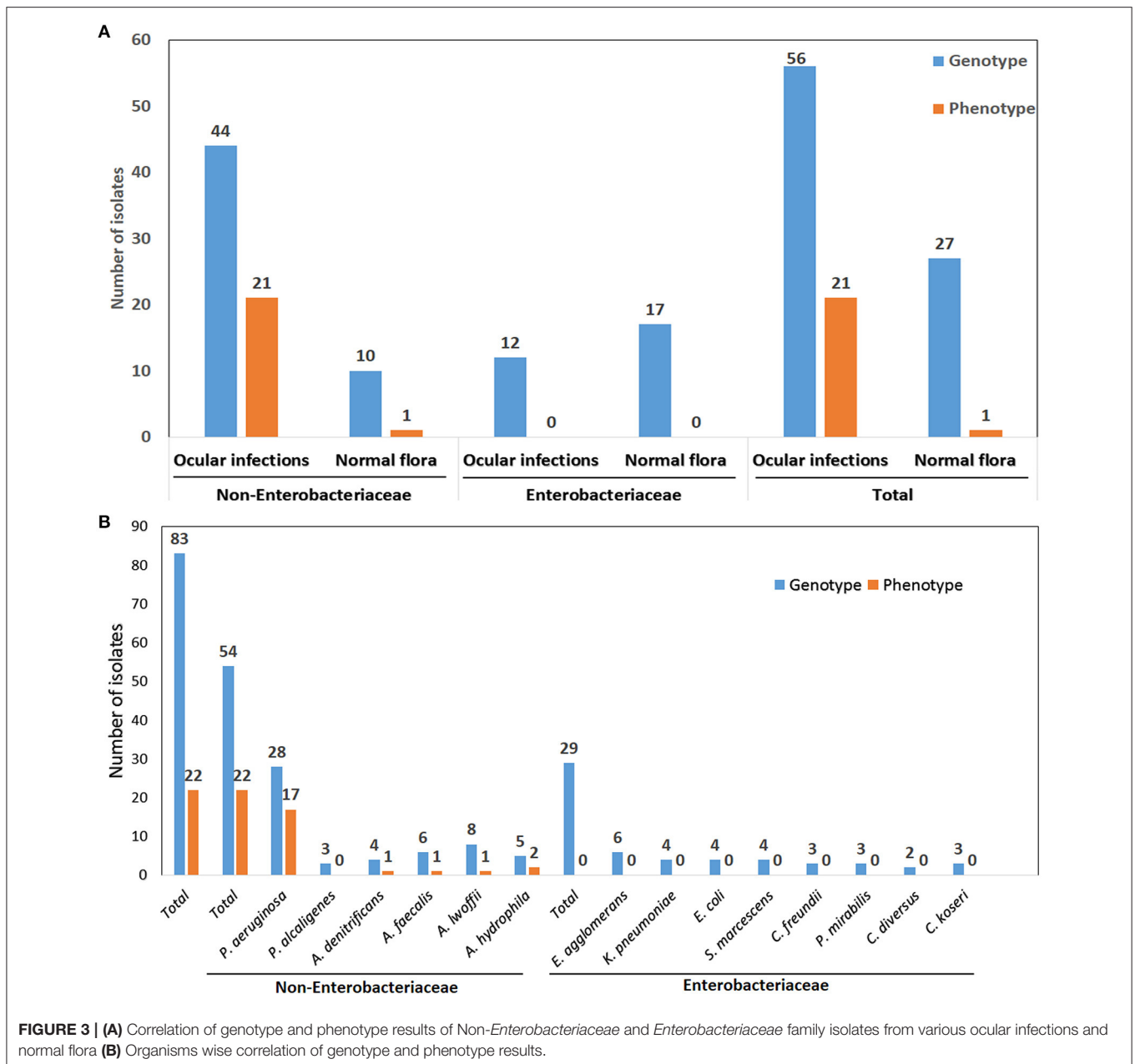
Bacterial pathogens recovered from various ocular infections showed a high level of susceptibility to chloramphenicol and meropenem, followed by amikacin, gatifloxacin, and ofloxacin, while a high level of resistance was noted against piperacillin as reported in previous studies (25). The mechanism of resistance to β-lactams antibiotics can be mediated by multiple mechanisms, but the β-Lactamases production is a major resistance determinant in the gram-negative bacteria (26). The β-lactamase confers resistance to all β-lactam antibiotics and is susceptible to monobactam antibiotics, although inhibited by metal chelators like EDTA (27). The development of resistance can also occur via acquisition of plasmids or mobile genetic elements like integrons, which often co-exist with other resistance determinants (28). MβL producers have been found in a variety of health-care settings around the world, particularly in high-risk areas like intensive care units (ICUs), surgical wards, neonatal ICUs, and bone marrow transplantation units (28). Among the MβLs, the most prevalent types are class B and subgroup 3a beta-lactamases which include the plasmid-mediated genes of *bla_{IMP}* and *bla_{VIM}* found globally and frequently among non-fermenting bacteria, including *Enterobacteriaceae* (12).

There have been sporadic reports of MβLs from major hospitals in India, with some of them reporting prevalence rates as high as 75% (29). In systemic infection, *P. aeruginosa* (16–94.2%) and *Acinetobacter* spp. (14.2–70.9%) are the most common pathogens (19, 21, 29, 30). Similarly, the current study also identifies a high prevalence of MβLs producing *P. aeruginosa* (81%), and 4.8% of *Acinetobacter lwoffii* from eye infections. Previous finding on the MβLs producing ocular *Enterobacteriaceae* have shown 8%, which is higher when compared with the recent study from India presenting only 1.25% from ICUs and surgical wards (31). In this study, we have not detected a MβLs producer among ocular *Enterobacteriaceae* family isolates from both infection and control groups. MβL producing *P. aeruginosa* isolates showed susceptibility to ceftazidime (30%) and gentamicin (37.5%) (29), 56% of resistance and 13% of intermediate resistance to imipenem (32). In the present study, MβL producing *P. aeruginosa* isolates showed 53% resistance to imipenem and 100% resistance to meropenem.

TABLE 4 | Antibiotic resistant profile of MBL vs non-MBL producing *P.aeruginosa* and other *Enterobacteriaceae* vs non-*Enterobacteriaceae*.

Name of the antibiotic agents	Infection group <i>P. aeruginosa</i>				Control group* <i>P. aeruginosa</i>				Infection group Non- <i>Enterobacteriaceae</i> (Other than <i>P. aeruginosa</i>)				Control group* Non- <i>Enterobacteriaceae</i> (Other than <i>P. aeruginosa</i>)				Non-MβL producing <i>Enterobacteriaceae</i>			
	MBL +ve n = 17		MBL -ve n = 69		MBL+ve n = 0		MBL -ve n = 22		MBL +ve n = 4		MBL -ve (n = 37)		MBL +ve n = 1		MBL -ve (n = 36)		Inf (n = 73)		Con (n = 100)	
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Carbapenems	17	100	8	12																
Meropenem (10 mcg)	17	100	8	12	0	0	5	23	4	100	3	8	1	100	3	8	4	5	10	10
Imipenem (10 mcg)	9	53	0	0	0	0	0	0	4	100	6	16	1	100	21	58	22	30	30	30
Fluroquinolones	17	100	13	19			0													
Levofloxacin (5 mcg)	17	100	13	19	0	0	4	18	1	25	5	14	0	0	2	6	20	27	19	19
Moxifloxacin (5 mcg)	16	94	13	19	0	0	4	18	1	25	6	16	0	0	4	11	24	33	25	25
Ofloxacin (5 mcg)	16	94	12	17	0	0	4	18	1	25	4	11	0	0	0	0	13	18	17	17
Ciprofloxacin (5 mcg)	15	88	12	17	0	0	3	14	1	25	4	11	0	0	2	6	23	32	20	20
Gatifloxacin (5 mcg)	15	88	11	16	0	0	4	18	1	25	4	11	0	0	2	6	14	19	18	18
Cephalosporins																				
2nd generation	17	100	69	100																
Cefoxitin (30 mcg)	17	100	69	100	0	0	22	100	4	100	35	95	1	100	34	94	30	41	22	22
Cefotetan (30 mcg)	17	100	69	100	0	0	22	100	2	50	33	89	1	100	17	47	12	16	10	10
3rd generation	17	100	69	100																
Cepfodoxime (10 mcg)	17	100	69	100	0	0	22	100	4	100	37	100	1	100	30	83	49	67	52	52
Ceftriaxone (30 mcg)	17	100	69	100	0	0	21	95	4	100	33	89	0	0	24	67	27	37	26	26
Cefotaxime (30 mcg)	17	100	40	58	0	0	10	45	4	100	17	46	1	100	12	33	31	42	26	26
Ceftazidime (30 mcg)	16	94	30	43	0	0	5	23	3	75	16	43	0	0	12	33	29	40	24	24
4th generation																				
Cefepime (30 mcg)	10	59	10	14	0	0	2	9	3	75	10	27	0	0	2	6	16	22	14	14
Monobactam																				
Aztreonam (30 mcg)	7	41	21	30	0	0	6	27	3	75	29	78	0	0	13	36	20	27	17	17
Peniciliins and its combinations	17	100	68	99																
Ticarcillin (75 mcg)	17	100	68	99	0	0	21	95	3	75	21	57	1	100	21	58	38	52	62	62

*Control group 96 Preoperative conjunctival flora.



The presence of the antibiotic resistant genes determines the prevalence of MβLs types, which varies greatly with geographical regions. In the present analysis, we found a high prevalence of 23% (83 of 359) *bla*_{VIM} type MβLs genes, with none representing *bla*_{IMP} and *bla*_{NDM} from preoperative conjunctival flora and various internal and external ocular infections. VIM-type enzymes were discovered in Verona, Italy, in 1997 (33), and 12 derivatives of the *bla*_{VIM-1} type of MβL gene have been reported worldwide to date (28). In terms of geographical distribution, the *bla*_{VIM} type gene has been found in *P. aeruginosa*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, and *E. coli* isolates from Europe, South East Asia, and North America, and, more recently, India, Iran, and Australia (28).

The current study revealed 17% (27 of 159) isolates carried the *bla*_{VIM} gene among the preoperative conjunctival flora,

while the remaining 28% (56 of 200) were the specimens obtained from the infected eye. In the current study, the *bla*_{VIM} gene was found in high abundance in *P. aeruginosa* and in low abundance in *Serratia marcescens*, *K. pneumoniae*, and *E. agglomerans*. Also, the current analysis highlights the presence of (*bla*_{VIM} type) MβLs mediated resistance genes among *P. aeruginosa* and Enterobacteriaceae family members. The high prevalence was detected predominantly in patients with keratitis and postoperative endophthalmitis, not with the normal flora. Compared to community and nosocomial-acquired infections, the prevalence currently determined is lower. (5).

There are multiple factors that determine the development of antibiotic resistance among the ocular isolates. The major concern is the use of higher antibiotic concentrations for treating ocular and preoperative prophylaxis than systemic

antibiotics (34). The widespread use of broad spectral antibiotics, especially in non-infectious ocular cases, promotes the rate of selection pressures in antibiotic resistance (35), thus altering the characteristics of bacterial pathogens (36).

CONCLUSION

To conclude, our findings fortified the fact that prevalence of the high resistance among the normal flora suggest the prophylactic measure is essential thus warranting a precisioned treatment in post-surgical period. Also, significant numbers of isolates having MβLs production also possess multidrug resistance. The emergence of MβLs-mediated resistance among ocular pathogens is a major problem for ophthalmologists since it limits the treatment options for treating ocular infections. Our study is only a preliminary report on the MβLs producing ocular gram-negative isolates from preoperative conjunctival flora and ocular infections, but it highlights the need for active surveillance. Large-scale multi-centric studies are required to understand the true nature of the prevailing antibiotic resistance pathogens. Monitoring the presence of drug resistance with detailed molecular prevalence will provide vital information for surveillance and help in evading these emerging mechanisms of antibiotic resistance. With the implication of advanced molecular methods like next generation sequencing, they will shed light on not only underlying molecular mechanisms but also aid in the development of better therapeutic targets.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

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ETHICS STATEMENT

This study was approved by Aravind Eye Hospital Institutional Ethics Committee.

AUTHOR CONTRIBUTIONS

GR and VP researched data, wrote manuscript, and contributed to discussion. RD researched data and contributed to discussion. PL, ST, AA, and FS contributed to discussion and reviewed/edited manuscript. SR and MZ researched data, contributed to discussion, and reviewed/edited manuscript. TA reviewed/edited manuscript. PA and FA researched data and reviewed/edited manuscript. FH researched data, wrote manuscript, and reviewed/edited manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2022.870354/full#supplementary-material>

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