CHARACTERIZATION OF ANTIBIOTIC RESISTANCE PROFILES OF OCULAR ENTEROBACTERIACEAE ISOLATES

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Emergence of extended-spectrum β-lactamase (ESBL) and fluoroquinolone resistance among ocular Enterobacteriaceae is increasing in higher frequency. Therefore, studies are being carried out to understand their multidrug resistance pattern. A total of 101 Enterobacteriaceae isolates recovered from various ocular diseases in a tertiary eye care center at Chennai, India during the period of January 2011 to June 2014 were studied. Forty one randomly chosen isolates were subjected to antibiotic susceptibility by minimum inhibitory concentration (MIC) and genotypic analysis. Of them, 16 were ESBL producers, one was carbapenemase producer and four were resistant to ertapenem which could be due to porin loss associated with AmpC production, and 17 were resistant to fluoroquinolones. Sixteen isolates harbored ESBL genes in which 14 had more than one gene and none of them were positive for *blaNDM-1* gene. *QNR* genes were detected in 18 isolates.

ESBL producers were predominantly isolated from conjunctiva. A high degree of ESBL production and fluoroquinolone resistance is seen among the genus *Klebsiella* sp. Hence, monitoring the rate of ESBL prevalence plays a vital role in the administration of appropriate intravitreal antibiotics to save the vision and also to reduce the development of drug resistance in ocular pathogens.

Keywords: ocular Enterobacteriaceae, multidrug resistance, extended-spectrum β-lactamase, fluoroquinolones, minimum inhibitory concentration, gene, prevalence

Introduction

The eye, an organ of the human body, is impermeable to almost all external infectious agents, though the ocular surface invariably is exposed to a wide array of microorganisms. Microbial infection of the eye is via external sources or through intraocular invasion of micro-organisms that are disseminated from the bloodstream or due to a breach in the ocular barriers. Alteration of the normal flora contributes to varied ocular diseases, including blepharitis, conjunctivitis, canaliculitis, dacryocystitis, orbital cellulitis, keratitis, and, finally, infectious endophthalmitis, including panophthalmitis. The most common microorganisms causing ocular infections include *Staphylococcus aureus,* coagulase negative staphylococci, genus Streptococcus, Corynebacterium, Bacillus, Nocardia, *Pseudomonas aeruginosa*, Enterobacteriaceae, nonfermenters, and others.

Despite the protection by the components of tear along with blinking action of the eyelids, the resident bacteria of the conjunctival sac or environmental bacteria can establish infection, resulting in the need for antibiotic intervention to treat the disease. Knowledge of the antimicrobial resistance, especially to commonly used antibiotics, is very important. Hence, potentially sight-threatening infections can emerge if appropriate antibiotic therapy is not instituted rapidly.

Antibacterial resistance has transpired as a major human health problem. The most common antibiotic resistances emerging among Enterobacteriaceae are caused by the β-lactamase enzymes that confer resistance to β-lactam

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antibiotics and have emerged over the last few decades to a greater extent owing to the antibiotic selection pressure. Of them, the most alarming are the extended-spectrum β-lactamase (ESBL) and carbapenemase-producing gram-negative bacilli [1], and these enzymes are encoded by point-mutational variants of classical broad-spectrum β-lactamases, which are referred to as ESBLs [2]. Different types of ESBLs are encountered in clinical settings worldwide, and the most important among them are *TEM-* and *SHV*-type and *CTX-M* β-lactamases. Other β-lactamases include metallo enzymes and *AmpC-* and *OXA*-type enzymes. Among the different β-lactamases, certain class A enzymes like KPC variants, NMCA, and SME-1; class B enzymes like IMP, VIM, and NDM variants; and class D – Oxa-type variants are able to hydrolyze carbapenems. The type of ESBL can be identified phenotypically using respective inhibitors by disk diffusion methods, and the carbapenemase production can be identified by modified Hodge test.

Fluoroquinolones (FQ) are among the most commonly prescribed antibiotics in outpatient and inpatient settings. Increases in the use of FQ in recent years have coincided with steady increase in the incidence of FQ resistance among gram-negative bacteria in intensive care units. Mechanisms involved in quinolone resistance are classically chromosome mediated [3], yet the last two decades have witnessed the emergence of plasmid-mediated quinolone resistance at higher frequency [4]. Multidrug-resistance carrying plasmid was discovered to encode transferable resistance to quinolones, along with reports on the existence of ESBL genes in them. Ability of resistance plasmids to get transferred among different bacterial species is of clinical importance, as it contributes to the increase in rate of antibiotic resistance in hospitalized patients [5]. Over the last 20 years, many β-lactam antibiotics that were specifically designed to resist the hydrolytic action of β-lactamases have been developed. However, with each new β-lactam agent introduced into the clinical practice, the capability of the bacteria to produce specific β-lactamases against the agent is also emerging in the same frequency. Therefore, antibiotic therapy is limited to a small number of expensive antibiotics which in turn has resulted in the emergence of newer ESBL and carbapenemase types in various species of the Enterobacteriaceae family.

Hospitalized patients are the main carriers of multidrug-resistant isolates [6]. Extensive studies on the resistance pattern of systemic multidrug resistant isolates which were FQ-resistant and ESBL producers exist, but similar studies in ocular isolates are limited [7]. Interestingly, infections caused by ESBL-producing, quinolone-resistant isolates are increasing in higher frequency among the ocular isolates [8]. Understanding the susceptibility pattern of ESBL-producing and carbapenemase-producing ocular Enterobacteriaceae isolates and their genotypic prevalence is, therefore, vital. It is equally important to study the quinolone resistance exhibited by the ESBL-resistant pathogens.

The factors contributing to development of drug resistance among ocular isolates include overuse of antibiotics for systemic infection as well as overuse of antibiotics in the eye. As a result, periodic susceptibility testing along with molecular biologic techniques for the detection of emerging antibiotic resistance among clinical isolates of bacteria is warranted to understand the prevalence of drug resistance to higher levels of drugs, and also to ensure the availability of broad-spectrum antimicrobials. With this background, this study aims to determine the ESBL pattern of resistance in ocular Enterobacteriaceae isolates and also to analyze the coexistence of quinolone resistance in them.

Materials and methods

Clinical specimens and bacterial strains

One hundred and one Enterobacteriaceae isolates were recovered from a total of 12,371 prospective ocular clinical specimens (including extraocular and intraocular) from patients with clinical diagnosis of bacterial ocular diseases, conjunctivitis, canaliculitis, keratitis, dacryocystitis, endophthalmitis, and panophthalmitis, along with swabs from donor corneal rim (DCR) and multiorgan donors (MOD) received at a tertiary eye care center in Chennai, India during the period January 2011 to June 2014. Among them, 41 isolates were further subjected to antibiotic susceptibility by agar dilution method and genotypic analysis. Only the first clinical isolate and/or admission screen for each patient was included. The study protocol was approved by the institutional ethics subcommittee of the hospital. Non-ESBL producer *Escherichia coli* ATCC 25922 was used as a negative control and ESBL producer *Klebsiella pneumoniae* ATCC 700603 was used as a positive control for the detection of ESBLs (ATCC; Manassas, VA, USA). Identification of these isolates was carried out with automated identification method using ID kits – mini API (Biomerieux, France), and the details of specimen-wise distribution of Enterobacteriaceae isolate included are given in *Table 1*. The primary screening for antibiotic susceptibility of the isolates was determined by disk diffusion method [9] against amikacin, ceftazidime, cefotaxime, norfloxacin, ofloxacin, ciprofloxacin, gentamicin, and tobramycin (Hi-Media Laboratories Private Limited, Mumbai, India).

Antibiotic susceptibility testing

Antibiotic susceptibility testing of the isolates was carried out by agar dilution method as per Clinical and Laboratory Standards Institute (CLSI) guidelines [9] to determine their pattern of resistance against β-lactams and FQs. Only for *Proteus* sp., it was done by broth microdilution method due to the swarming nature of this species on agar plates. β-lactam antibiotics tested include

Table 1. Distribution of 41 ocular Enterobacteriaceae isolates included in the present study

Organism	No. of isolates (n = 41)	Details of ocular clinical specimens		
		Intraocular	Extraocular	Others
K. pneumoniae	11 (26.8%)	Eviscerated material (n = 1)	Conjunctival swab (n = 2), orbital biopsy (n = 1), corneal scraping (n = 1), corneal button (n = 1)	Contact lens (n = 3), donor corneal rim (DCR) (n = 2)
K. oxytoca	8 (19.5%)	Eviscerated material (n = 1)	Conjunctival swab (n = 1), corneal scraping (n = 1), canalicular pus (n = 1), orbital biopsy (n = 1)	Contact lens (n = 1), donor corneal rim (DCR) (n = 2)
E. aerogenes	7 (17.0%)	Aqueous humor (n = 1), vitreous humor (n = 1)	Conjunctival swab (n = 2), corneal scraping (n = 2)	Donor corneal rim (DCR) (n = 1)
S. marscensens	6 (14.6%)	Vitreous humor (n = 1)	Corneal scraping (n = 1), iris tissue (n = 1)	Contact lens (n = 2), bandage contact lens (n = 1)
C. freundii	3 (7.3%)	0	Conjunctival swab (n = 1)	Contact lens (n = 1), swab from multiorgan donor (MOD) (n = 1)
E. coli	3 (7.3%)	Vitreous humor (n = 1), intraocular lens (n = 1)	Conjunctival swab (n = 1)	0
C. diversus	1 (2.4%)	0	0	Swab from multi-organ donor (MOD) (n = 1)
P. mirabilis	1 (2.4%)	0	Corneal scraping (n = 1)	0
M. morganii	1 (2.4%)	0	Corneal scraping (n = 1)	0

amoxicillin, piperacillin, ceftazidime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, ertapenem, and doripenem (Orchid Chemicals and Pharmaceuticals Limited, Chennai, India). For the identification of ESBLs, the β-lactam antibiotics were tested in combination with the β-lactamase inhibitors (BLIs), namely, amoxicillin with clavulanic acid (2:1 ratio) (Fluka, Sigma-Aldrich, Germany), piperacillin with tazobactam (4 μg fixed concentration) (Orchid Chemicals and Pharmaceuticals Limited, Chennai, India), and ceftazidime and cefotaxime with clavulanic acid (4 μg fixed concentration). The isolates were also tested for their susceptibility against FQs which includes ciprofloxacin, levofloxacin, and gatifloxacin by agar dilution method. The presence of carbapenemase was identified by the modified Hodge test (MHT) as described by Center for Disease Control and Prevention (http://www.cdc.gov/HAI/pdfs/labSettings/HodgeTest_Carbapenamase_Enterobacteriaceae.pdf).

Polymerase chain reaction (PCR) for the detection of ESBLs, AmpC, NDM, QNR, and gyrase genes

PCRs corresponding to *CTX-M* genes (for all four groups) [10, 11] *TEM, OXA, AMPC, SHV* [12], *NDM*-1, *gyrase A, gyrase B* [13], and *QNR* genes [14] were carried using the primers and protocol as described in *Table 2*. Sensitivity of PCRs ranges from 10 ng to 50 ng DNA of ESBL-producing positive controls. The primers were highly specific,

and no amplification was observed with fungal, viral, and human DNA.

DNA sequencing reaction

All PCR positive amplified products were subjected to DNA sequencing with the primers of the corresponding genes, and the results were analyzed as described earlier [8].

Ethical approval

It was submitted to institutional ethics subcommittees (IRB) No. 95-2007P.

Results

Primary screening of the isolates by disk diffusion method

All the 41 clinical Enterobacteriaceae isolates were subjected to routine antibiotic susceptibility testing, and the results were as follows: among them, 25% were resistant to amikacin, 41% to ceftazidime, 44% to cefotaxime, 30% to norfloxacin, 30% to ofloxacin, 34% to ciprofloxacin, 37% to gentamicin, and 14% to tobramycin.

Table 2. The gene targets, their corresponding primers, and thermal profiles

Primers	Primer sequence (5′–3′)	Product size (bp)	PCR thermal profile	Reference
CTX-M group I – F	5′-GACGATGTCACTGGCTGAGC-3′	499	Initial denaturation at 96 °C for 15 s; 24 cycles at 96 °C for 15 s, 55 °C for 15 s, and 72 °C for 2 min; final extension: 72 °C for 10 min	Johann et al.
CTX-M group I – R	5′-AGCCGCCGACGCTAATACA-3′			
CTX-M group II – TOHO-F	5′-GCGACCTGGTTAACTACAATCC-3′	351	Initial denaturation at 96 °C for 15 s; 24 cycles at 96 °C for 15 s, 55 °C for 15 s, and 72 °C for 2 min; final extension: 72 °C for 10 min	Johann et al.
CTX-M group II – TOHO-R	5′-CGGTAGTATTGCCCTTAAGCC-3′			
CTX-M group III – F	5′-CGCTTTGCCATGTGCAGCACC-3′	307	Initial denaturation at 96 °C for 15 s; 24 cycles at 96 °C for 15 s, 55 °C for 15 s, and 72 °C for 2 min; final extension: 72 °C for 10 min	Johann et al.
CTX-M group III – R	5′-GCTCAGTACGATCGAGCC-3′			
CTX-M group IV – F	5′-GCTGGAGAAAAGCAGCGGAG-3′	474	Initial denaturation at 96 °C for 15 s; 24 cycles at 96 °C for 15 s, 62 °C for 15 s, and 72 °C for 2 min; final extension: 72 °C for 10 min	Johann et al.
CTX-M group IV – R	5′-GTAAGCTGACGCAACGTCTG-3′			
TEM – F	5′-ATGAGTATTCAACATTTCCG-3′	850	Initial denaturation at 94 °C for 5 min; 2 cycles at 94 °C for 7 min, 60 °C for 5 min, and 72 °C for 1 min; followed by 30 cycles with 94 °C for 1 min, 60 °C for 2 min, and 72 °C for 1 min; final extension: 72 °C for 10 min	Eckert et al.
TEM – R	5′-CCAATGCTTAATCAGTGA GG-3′			
OXA – F	5′-TATCTACAGCAGCGCCAGTC-3′	199		Constanca et al.
OXA – R	5′-CGCATAAATGCCATAAGTG-3′			
SHV – F	5′-TCAGCGAAAAACACCTTG-3′	475		M'Zali et al.
SHV – R	5′-TCCCGCAGATAAATCACCA-3′			
AMPC – F	5′-CCCCGCTTATAGAGCAACAA-3′	634		Constanca et al.
AMPC – R	5′-TCAATGGTCGACTTCACACC-3′			
QnrAm-F	5′-AGAGGATTTCTCACGCCAGG-3′	580	Initial denaturation at 95 °C for 10 min; 35 cycles at 95 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min; final extension: 72 °C for 10 min	Cattoir et al.
QnrAm-R	5′-TGCCAGGCACAGATCTTGAC-3′			
QnrBm-F	5′-GGMATHGAAATTCGCCACTG-3′	264		
QnrBm-R	5′-TTTGCYGYYCGCCAGTCGAA-3′			
QnrSm-F	5′-GCAAGTTCATTGAACAGGGT-3′	428		
QnrSm-R	5′-TCTAAACCGTCGAGTTCGGCG-3′			
NDM-F	5′-TCTCGACATGCCGGGTTTCGG-3′	475	Initial denaturation at 94 °C for 10 min; 30 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min; final extension: 72 °C for 10 min	Sowmiya et al.
NDM -R	5′-ACCGAGATTGCCGAGCGACT-3′			

Phenotypic results: antibiotic susceptibility of the isolates by minimum inhibitory concentration (MIC)

Susceptibility to β-lactams

Among the 41 Enterobacteriaceae isolates tested against β-lactam antibiotics and their combinations, 16 isolates produced ESBLs, which was evident from decrease in MIC of ceftazidime and cefotaxime by ≥3 two-fold concentrations in the presence of clavulanic acid. One *Enterobacter aerogenes* isolate was resistant to the tested carbapenems, and four isolates were intermediately resistant to ertape-nem (MIC of 1–2 mg/l) while showing susceptibility to imipenem, meropenem, and doripenem. The resistance pattern is shown in *Fig. 1*.

Susceptibility to fluoroquinolones

The isolates were classified as FQ resistant based on the CLSI guideline break points for ciprofloxacin, levofloxacin, and gatifloxacin. Among 41 isolates, 13 were resistant to FQ with MIC ranging from 8 mg/l to ≥16 mg/l, and the remaining 28 isolates were susceptible as shown in *Fig. 2*.
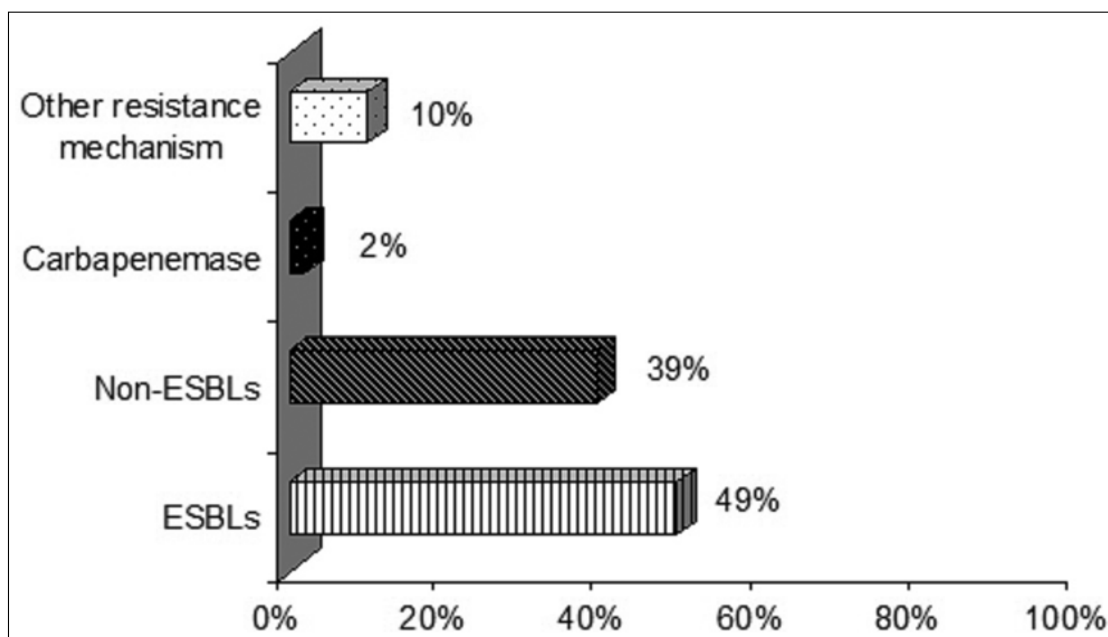
Fig. 1. Resistance pattern of isolates against β-lactams

Antibiotic group-wise resistance pattern

Antibiogram against β-lactams showed 16 isolates as ESBL producers, and one *E. aerogenes* isolate from DCR showed carbapenemase production, which was confirmed by modified Hodge test (MHT). Four isolates showed intermediate resistance to ertapenem with a moderately elevated MIC (1–2 mg/l) while showing negative for MHT, indicating that other mechanisms of resistance like porin loss along with AmpC and 20 isolates were non-ESBL producers. Among the 13 FQ-resistant isolates, nine were ESBL producers whereas one was resistant to carbapenem and the remaining three were non-ESBL producers *(Fig. 3)*. Among the 28 FQ-susceptible isolates, seven were ESBL producers and the remaining four were resistant by other mechanisms while the remaining 17 isolates were non-ESBL producers.
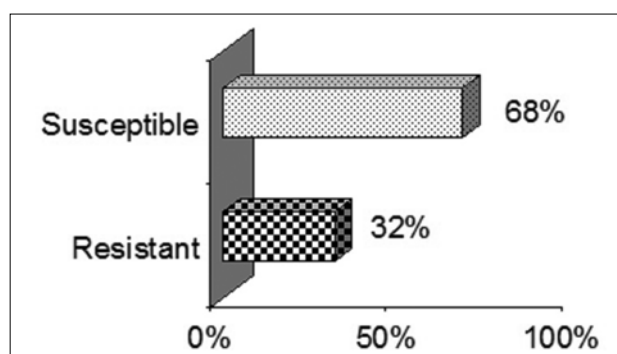

Fig. 2. Resistance pattern of isolates against fluoroquinolones

Bacterial species-wise resistance pattern

Majority of ESBL producers belonged to *K. pneumoniae* (six isolates) followed by *Klebsiella oxytoca* and *E. aero-*
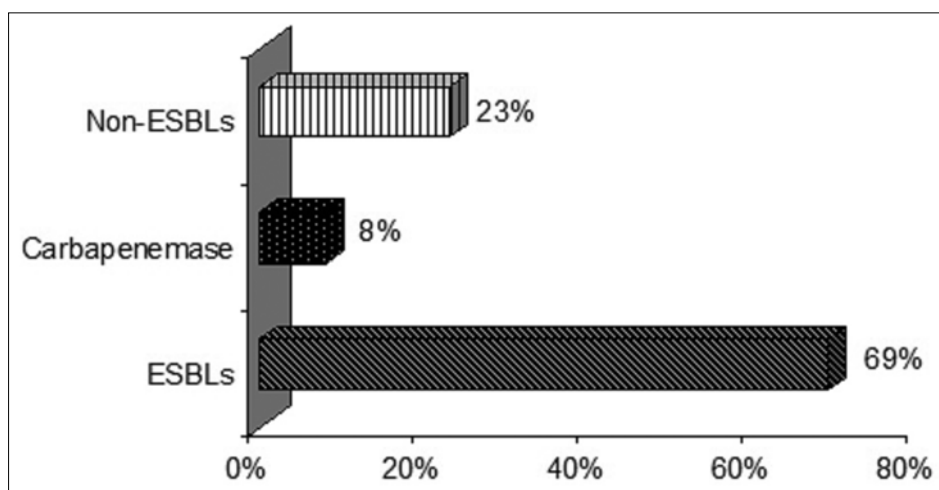

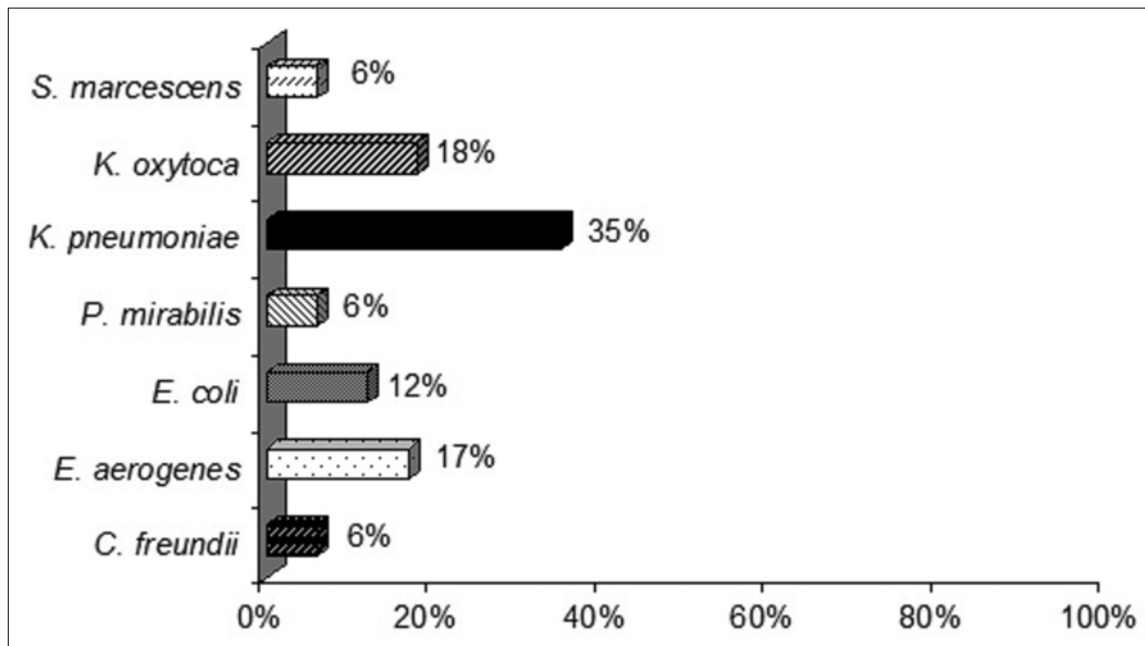**Fig. 3.** β-lactam resistance pattern of FQ-resistant isolates

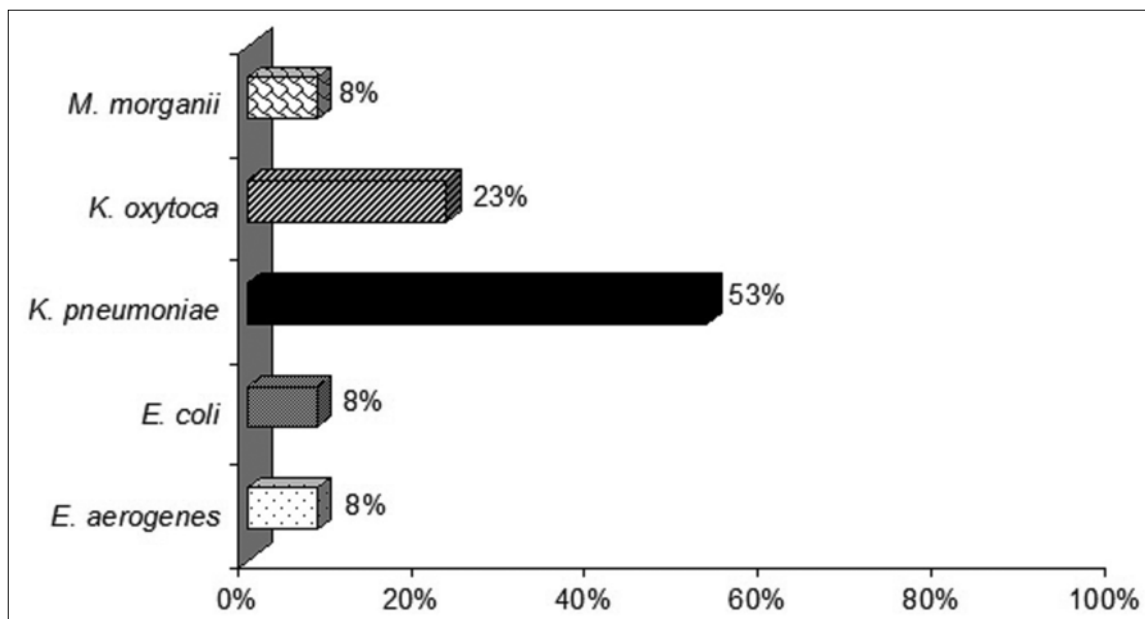**Fig. 4.** β-lactam resistance across the genus

Fig. 5. FQ resistance across the genus

genes (three isolates each), *E. coli* (two isolates) and *Citrobacter freundii,* and *Serratia marcescens* and *Proteus mirabilis* (one isolate each) *(Fig. 4)*. FQ resistance was observed in seven *K. pneumoniae* isolates followed by three isolates of *K. oxytoca* and one isolate each of *E. coli, E. aerogenes,* and *Morganella morganii (Fig. 5)*.

Genotypic results

Genotypic results of ESBLs, AmpC, and NDM genes detection among Enterobacteriaceae

Out of 41 Enterobacteriaceae isolates subjected to ESBL detection by the molecular method, 16 isolates showed the presence of ESBL genes, which matched the phenotypic detection results. Of the 16 isolates, two isolates had single gene positivity; one for *blaCTX-M* and other for *blaOXA*. Four isolates harbored two ESBL genes, each of *blaOXA + AmpC, blaOXA + blaCTX-M, blaOXA + blaSHV,* and *AmpC + blaOXA*. Eight isolates carried three ESBL genes, each of *blaCTX-M + blaTEM + blaOXA, blaCTX-M + blaTEM + AmpC,* and *blaCTX-M + AmpC + blaOXA*. Two isolates had four gene combinations, i.e., *blaCTX-M + blaOXA + AmpC + blaTEM,* and the remaining 22 isolates were found to be susceptible to all β-lactams tested. *blaNDM*-1 gene was absent in the carbapenem-resistant isolate.

Genotypic result of QNR genes detection among Entero-bacteriaceae isolates

All 41 Enterobacteriaceae isolates were subjected to QNR detection by molecular method, and 18 isolates showed the presence of *QNR* genes. Of the 18 QNR producers, eight isolates showed positive *qnrA* gene alone, seven isolates showed positive *qnrB,* two isolates showed positive for both *qnrA* and *qnrB* (dual gene positive), and one isolate showed *qnrS* positive (single gene positive). Among the isolates which showed resistance to FQ by phenotypic detection, 12 were found to be positive for *qnrA* or *qnrB* or both and one was positive for *qnrS*. One isolate which showed the presence of *qnrA* gene alone and four isolates which showed the presence of *qnrB* gene was found to be susceptible for the FQs tested. This result suggests that, even though the gene is present, the resistance is not reflected in the phenotypic detection by MIC.

Genotypic results of FQ-resistant ESBL-producing Enterobacteriaceae

Among 18 FQ-resistant isolates, four were *CTX-M-15* positive, one in each was *AmpC* and *OXA* positive, six others were positive to both *CTX-M-15* and *AmpC,* four were positive for *CTX-M-15* and *OXA,* and four produced all three enzymes apart from being resistant to FQ. Four of the non-ESBL producers were resistant to FQ. Out of 23 FQ-susceptible isolates, two isolates coproduced *CTX-M-15, AmpC,* and *OXA* enzymes, and four were ertapenem resistant. The rest of the FQ-susceptible isolates were susceptible to all β-lactams.

Sequencing result of gyrase genes detection among Enterobacteriaceae isolates

All the 41 Enterobacteriaceae isolates were subjected to the detection of mutation in the *gyrase A* and *gyrase B* genes by molecular method. In case of mutations among *gyrase A* gene, a total of 19 isolates had point mutation in Ser83 (Ser83Leu) in 18 isolates, Ser83Ile in one isolate, and one isolate had dual mutation in both Ser83Tyr and Asp87Ala. None of the isolates showed mutation in *gyrase B* gene. All the 10 isolates which were positive for *qnrA* gene were also positive for *gyrase A* gene.

Nucleotide sequence accession number

The nucleotide sequence data reported in this paper will appear in the GenBank nucleotide sequence database under accession number: CTX-M-15 – KC528758–KC528772, OXA-1 – KC528773–KC528778, SHV-1 – KC528783–KC528789, TEM-1 – KC528790–KC528796 and JN043381–JN043382, *QNR*-A-1 – JQ235817–JQ235818, *QNR*-B-1 – KC528779–KC528782, and AMPC – JN043374, JQ235790–JQ235791.

Discussion

The resistance to extended-spectrum cephalosporins is mainly mediated by the production of ESBLs. A number of nosocomial outbreaks caused by ESBL-producing organisms has been reported in several parts of the world. Although most of the outbreaks were limited to high-risk patient care areas such as ICUs, oncology units, etc., the first report of an outbreak in nursing homes appeared in the literature in the year 1999 [15].

Fluoroquinolone resistance in clinical isolates of Enterobacteriaceae species has been reported with increasing frequency in recent years [13, 17]. Two mechanisms of FQ resistance have been identified in gram-negative organisms: mutations in DNA gyrase and reduced intracellular drug accumulation. Weigel et al. [13] showed that a single-point mutation in *gyrA* has been shown to reduce the susceptibility to FQ. The FQ-resistant enterotoxigenic *E. coli* strains had mutations in *gyr*A at positions 83 and 87. The *gyrA* mutations that frequently affect residue serine at 83 and aspartate at 87 are common among FQ-resistant *E. coli*. In addition to the mutations in *gyrA,* mutations were detected in *parC*. For the expression of high-level resistance, acquisition of a second *gyrA* mutation and a *parC* mutation seems important. A similar resistance mechanism has been observed in *E. coli* strains in our study, and it was also described by Heisig et al. [16] and Pazhani et al. [17]. Similarly, in our study, the mutations were observed in *gyrA* at positions 83 and 87.

Oethinger et al. [18] observed that the absence of *AcrAB* efflux pump in *E. coli* and *gyrA* mutations hardly increase the MICs of quinolones. Even with a functional efflux system, single mutations in *gyrA* produce only a modest increment in resistance, such that they would be considered to be clinically susceptible (i.e., the MIC of ciprofloxacin is ≤1 mg/l). Only with a second mutation in *gyrA* or a mutation in *parC,* a clinical level of resistance (i.e., an MIC of ≥4 mg/l) is reached [19]. In general, the more resistant a clinical isolate to commonly used drugs, the more chance of it to have quinolone resistance-associated mutations.

We have found that 32% of the isolates were resistant to FQ and mutations were confirmed genotypically. Five isolates which were genotypically positive for *QNR* gene had lower MIC values. This result was in correlation with the observations of Briales et al. [20] as it could be due to the presence of any qnr gene and absence of other resistance mechanisms.

In summary, among the chosen 41 isolates, 16 strains were ESBL producers, one strain was a carbapenemase producer. Among the 13 FQ-resistant isolates, nine were ESBL producers and one was a carbapenemase producer. This shows the scenario of increasing pattern of ESBL producers possessing FQ resistance among Enterobacteriaceae isolates especially among *K. pneumoniae, E. aerogenes,* and *E. coli*.

Our study also showed the absence of blaNDM-1 genes among ocular specimens. This demonstrates that,

at this time point, blaNDM-1 genes are not carried among the organisms isolated from extraocular specimens that are highly exposed to community-acquired infections and also from intraocular specimens, making them free from increased drug resistance and, thus, making the treatment not a hard task. However, the high rates of ESBL-producing isolates among ocular strains are an important finding. Cefazolin and cefotaxime are being used routinely in the treatment of ocular infections. Considering the increasing spread of such ESBL genes in the community, all patients with a culture-positive report should carry a note indicating whether the isolate is an ESBL producer or not. This will help clinicians to avoid use of penicillins, aztreonam, and all cephalosporins (except cephamycins), irrespective of their *in vitro* susceptibility and save the last resort antibiotics for future.

The detection of ESBL production by the isolates is time consuming, but still, it has high value in treating severely ill patients, septicemic patients, and young children and in case of ocular infections especially in one-eyed patients, post-operative endophthalmitis patients, and trauma cases. Since the FQs were mostly used to treat ocular infections, it is an important finding that the FQ resistance among the isolates has to be tested prior to the initiation of antibiotic therapy, and so the treatment of ocular infections is becoming a challenge in the phase of antibiotic resistance among the ocular pathogens. Hence, proper identification of the resistant bacterial pathogens and the treatment with appropriate antibiotics should be carried out to avoid the development and spread of more resistant ocular pathogens in the future.

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Conflicts of interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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