



**ORIGINAL RESEARCH PAPER**

**Ophthalmology**

**EMERGING FLUOROQUINOLONE RESISTANCE IN OCULAR PATHOGENS.**

**KEY WORDS:**  
Fluoroquinolones, disc diffusion method, csi guidelines, resistance.

<b>Dr.Ashok Kumar Meena</b>	Professor and Head, Department of Ophthalmology, Government Medical College, Kota.
<b>Dr.Pushpendra Chotiya*</b>	P G Resident Final Year, Department of Ophthalmology, Government Medical College, Kota. *Corresponding Author
<b>Dr.Jaishree Singh</b>	Professor, Department of Ophthalmology, Government Medical College, Kota.

**ABSTRACT**

**Introduction:** The fluoroquinolone cover a host of Gram –negative, Gram-positive microorganisms and anaerobes. Incidence of in vitro resistance to older Fluoroquinolone increasing because of misuse, inappropriate dosing and schedule.  
**Methodology:** Study was done on 100 patients suspected of bacterial ocular infections. Cultures were performed, sensitivity testing done by disc diffusion method. Grades were recognized sensitive, intermediate, resistant by comparing the diameters of inhibition zone according to CLSI guidelines. Eyes were treated by eye drops of older group including ciprofloxacin 0.3%, ofloxacin 0.3%, and newer group including gatifloxacin 0.3%, moxifloxacin 0.5%, besifloxacin 0.6%.  
**Conclusion and results:** In this prospective study, it was found that resistance against newer fluoroquinolones is less in comparison to older. Bacterial drug resistance can be reduced by proper use of antibiotics, cycling of different antibiotics in chronic cases, culture and sensitivity study in serious infections and use of fluoroquinolones on the basis of susceptibility profiles, using mutants prevention concentration.

**Introduction-**

Bacteria are present as microbial flora of the external ocular surface. Several protective mechanisms operate on the eye surface and prevent eye infections. Breach in surface epithelium predispose the eye to bacterial infections. Bacteria causing conjunctivitis, scleritis, keratitis, Blepharitis, canaliculitis, dacryocystitis, orbital cellulitis, necrotizing fasciitis, uveitis, endophthalmitis etc. The causative bacteria are commonly staphylococcus, streptococcus and Haemophilus species. The fluoroquinolone represent broad spectrum antibacterial, which cover a host of Gram –negative, Gram-positive microorganisms and anaerobes. Incidence of in vitro resistance to older Fluoroquinolone increasing because of misuse, inappropriate dosing and schedule. Resistance among common ocular pathogens necessitates the development of new and novel antibiotics. Fluoroquinolone are classified as Newer include Gatifloxacin 0.3%, 0.5%, Moxifloxacin 0.5% and Besifloxacin 0.6%. Older include Ciprofloxacin 0.3% and Ofloxacin 0.3%. The most important attribute of Newer Fluoroquinolone is their enhanced gram positive activity relative to Ciprofloxacin & Ofloxacin. Fluoroquinolone inhibit DNA gyrase and Topoisomerase IV.<sup>1,2</sup>

Fluoroquinolone resistance develop through 2 main mechanisms:<sup>3</sup>

1. Alteration in drug target enzyme as mutation in DNA gyrase and mutations in topoisomerase IV.
2. Alteration in access to drug target enzyme by expression of Multidrug resistant MDR membrane associated efflux pumps, Which actively pump out of bacterial cells.

**MATERIAL AND METHOD**

This Prospective study was done on 100 outdoor and Indoor patients in department of ophthalmology, government medical college, kota from 2016 to 2017.

**INCLUSION CRITERIA**

All cases of bacterial ocular infections as conjunctivitis, Blepharitis, Keratitis, Corneal ulcer and Endophthalmitis.

**EXCLUSION CRITERIA**

Fungal corneal ulcer, Viral corneal ulcer, Any preexisting septic foci and viral conjunctivitis.

Cultures were performed from patients with informed consent. Susceptibility testing of each isolate was assessed clinically and in

laboratory to Fluoroquinolones. In vitro susceptibility testing was determined by Disc Diffusion method (KIRBY –BAUER)<sup>4</sup> and interpreted by using the national committee for clinical laboratory standards for studies. Clinically healing of corneal ulcer is confirmed by 2% sterile Fluorescein stain method. Single disc of standard content of antibiotics are placed on inoculum of strictly standardized density on Muller Hinton Agar. Three Grades of sensitivity were recognized sensitive, intermediate, resistant by comparing the diameters of inhibition zone with critical zone diameter according to CLSI guidelines.

**RESULTS AND DISCUSSION**

**Table -1 Incidence of Bacterial Ocular Disease in Various age groups**

S.No.	Age Group (in years)	N	Percentage
1	0-20	24	24 %
2	21-40	42	42%
3	41-60	22	22%
4	>60	12	12%

**Table -2 Sex Ratio Distribution**

Disease Group	Male		Female	
	N	%	N	%
Disease of Lacrimal Apparatus (Acute Dacryocystitis, Lacrimal Abscess)	2	2%	10	10%
Disease of Conjunctiva (Acute Conjunctivitis)	12	12%	8	8%
Disease of Cornea (Keratitis)	34	34%	16	16%
Miscellaneous (Stye, ulcerative blepharitis, perforating injury)	10	10%	8	8%
Total	58	58%	42	42%

**Table -3 Demographic Variation**

Disease Group	Rural		Urban	
	N	%	N	%
Disease of Lacrimal Apparatus (Acute Dacryocystitis Lacrimal Abscess)	2	2%	10	10%
Disease of Conjunctiva (Acute Conjunctivitis)	12	12%	8	8%
Disease of Cornea (Keratitis)	34	34%	16	16%
Miscellaneous (Stye, Ulcerative Blepharitis, Perforating injury)	10	10%	8	8%
Total	58	58%	42	42%

**Table -4 Distribution of Bacterial Isolates Identified**

Bacteria	Total	Percentage
Gram –Positive Bacteria	74	74%
Gram-Negative Bacteria	26	26%
Total	100	100%

**TABLE 5 SENSITIVITY AND RESISTANCE OF GRAM –POSITIVE BACTERIA TO FLUOROQUINOLONES (TOTAL NUMBER OF CASES, N-74)**

S no	Fluoroquinolones	Number of bacteria found SENSITIVE to fluoroquinolone by Kirby bauer disc diffusion method		Number of bacteria found RESISTANT to fluoroquinolone by Kirby bauer disc diffusion method	
		N	Percentage	N	Percentage
1	Ciprofloxacin	41	55.40	33	44.59
2	Ofloxacin	43	58.10	31	41.89
3	Gatifloxacin	56	75.67	18	24.32
4	Moxifloxacin	58	78.37	16	21.62
5	Besifloxacin	74	100	0	00

**TABLE 6 SENSITIVITY AND RESISTANCE OF GRAM –NEGATIVE BACTERIA TO FLUOROQUINOLONES (TOTAL NUMBER OF CASES, N-26)**

S no	Fluoroquinolones	Number of bacteria found SENSITIVE to fluoroquinolone by Kirby bauer disc diffusion method		Number of bacteria found RESISTANT to fluoroquinolone by Kirby bauer disc diffusion method	
		N	Percentage	N	Percentage
1	Ciprofloxacin	19	73.07	07	26.92
2	Ofloxacin	20	76.92	06	23.03
3	Gatifloxacin	21	80.76	05	19.23
4	Moxifloxacin	22	84.61	04	15.38
5	Besifloxacin	26	100	00	00

In our study the age of patients range from 5 days up to 73 years (Table No-1). The maximum number of patients of our study were in the age group of 21 to 40 years ,i.e. 42( 42%) out of total 100 patient .The higher incidence of bacterial ocular infections in younger age group appears to be due to their much more involvement in outdoor activities and thus more prone to trauma and excessive exposure to environmental risk factors, i.e.dry heat, dust, sunlight etc. For longer duration of time in comparison to older age group.

In our study (Table No-2)we found that males are slightly more involved 58( 58%) as compared to42( 42%)females due to more outdoor activity of male in comparison to female.

(Table No 3) shows that incidence of ocular infection in rural population 58% was more as compared to Urban population 42% since rural population is more exposed to trauma as rural population is agricultural based population having much more outdoor activity and therefore having higher chances of injury to eye as compared to Urban population .

In our research work as shown (Table No- 4) out of 100 in vitro - culture identified, 74 were Gram Positive Bacteria while 26 were Gram Negative bacterial isolates. The ratio of Gram Positive Bacteria to Gram Negative bacteria comes out to be 2.84 : 1 .Similar results are seen in a 5 years retrospective review study conducted by Goldstein et al<sup>5</sup> at the Charles T. Campbell ophthalmic microbiology laboratory at the Eye and Ear Institute ,Pittsburgh between 1993 to 1997. In their study, out of 1053 bacterial isolates 797( 75.7% ) were Gram positive bacteria and Gram Negative bacteria were 256( 24.3%) and the ratio between Gram positive and Gram Negative bacteria comes out to be 3.11 : 1 .Similar study conducted by Kowalski et al<sup>6</sup>, Aleandrakes et al<sup>7</sup>.

(TABLE No- 5) shows the comparison between all 5 fluoroquinolones among Gram Positive Bacteria ciprofloxacin showed resistance in 33 (44.59%) cases, ofloxacin in 31(41.89%) cases, gatifloxacin in 18 (24.32%)cases, moxifloxacin in 16(21.62%)cases, besifloxacin in zero case.

Thus it is concluded that 4th generation newer fluoroquinolones like besifloxacin 0.6%,moxifloxacin0.5%,gatifloxacin 0.3% has best activity against Gram Positive Bacteria and emergence of resistance to older fluoroquinolones like ciprofloxacin and ofloxacin was observed.

(TABLE No- 6) shows the resistance pattern in Gram Negative bacteria Ciprofloxacin showed resistance in 7 cases(26.92%), ofloxacin in 6(23.03%) cases ,gatifloxacin in 5 (19.23%)cases, moxifloxacin in 4(15.38%)cases, besifloxacin in zero case. The above table shows that there is no significant emergence of resistance for newer as well as older fluoroquinolones on the basis of laboratory and clinical evaluation .Thus ,older fluoroquinolones are still promoting for the treatment of gram negative bacterial ocular infections. Emerging resistance among ocular pathogens to older fluoroquinolones, particularly among Gram Positive organisms, had lead to the development of newer agents like gatifloxacin,moxifloxacin,besifloxacin. The addition of a methoxy side chain at the R8 position led to the development of 4th generation compounds like gatifloxacin. It also carries methyl group on the piperaziny! ring.Besifloxacin, a C8-chloro-fluoroquinolone newer fluoroquinolone.

Mathar et al<sup>8</sup>did in vitro study of 93 bacterial endophthalmitis cases and found that staphylococcus aureus and streptococcus viridans which were resistance to Ciprofloxacin and Ofloxacin were statistically more susceptible to Gatifloxacin (p<0.05). similarly PARMER et al found Gatifloxacin treated keratitis had exhibited complete healing compared with those of Ciprofloxacin treated group (p=0.042). Darlene et al<sup>9</sup> did study to see in vitro susceptibility and cross resistance of gatifloxacin and moxifloxacin to older fluoroquinolones among 111 coagulase –negative staphylococcus recovered from patients with clinical endophthalmitis during 15 year period (January 1,1990 to December 31 2004) demonstrated less than 80 %susceptibility to gatifloxacin and moxifloxacin. Haas et al<sup>10</sup>studied in 2690 clinical isolates representing 40 speceis 34 aerobic and 6 anaerobic bacterial species, the in vitro activity of besifloxacin was generally superior to that of existing agents used for topical treatment of ocular infections. The consistently improved activity profile of besifloxacin against gram-positive and gram-negative pathogens that were resistant to other fluoroquinolones was particularly notable. In conjunction with recently reported, besifloxacin's broad-spectrum activity profile is appropriate for empirical treatment of bacterial infections. Asbell et al<sup>11</sup> did multicenter prospective antibiotics resistance monitoring in ocular micro organism (ARMOR) study during 2009 to 2015 on around 4000 isolates found that besifloxacin, demonstrate significant in vitro activity against many resistant bacterial pathogens ,the resistance of Staphylococcus isolates to older fluoroquinolones.

**CONCLUSION**

1. In this clinico- microbiological study of ocular pathogens, it was found that there is emergence of resistance to older fluoroquinolones like Ciprofloxacin and Ofloxacin in ocular pathogens.
2. The resistance against the newer fluoroquinolones is less in comparison to older fluoroquinolones.
3. Bacterial drug resistance of fluoroquinolones can be reduced by proper use of antibiotics, cycling of different antibiotics in chronic cases, culture and sensitivity study in serious infections and use of fluoroquinolones on the basis of susceptibility profiles besides regulating over the counter (OTC) sale of drugs using mutants prevention concentration and modifying the dose regimes.

This interesting study has further scope of research in future development of newer fluoroquinolones and development of various alternative strategies like plasmid containing engineered DNA, antimicrobial peptides like protegrins, defensins, etc. immunotherapy with cytokines and phototherapy by using differential phototoxicity of photosensitizers.

**REFERENCES**

1. Blondeau JM. Expanded activity of the new fluoroquinolones: a review. ClinTherapeu, 1999; 3-40.

2. Zechiedrich EL, Cozzarelli NR. Roles of topoisomerases IV and DNA gyrase in DNA unlinking during replication in E. Coli. *Genes Dev*, 1995; 9: 2859-69.
3. Hooper DC. Mechanism of fluoroquinolone resistance . *Drug Resist Updates*, 1999; 2: 38-55.
4. Baurer AW, Kirby WMM, Shersis JC, Turck M. Antiviotic susceptibility testing by a standardized single disk method. *Am J Clinical Pathology* 1996; 45: 493-496.
5. Goldstein MH, Kowalski RP et al Emerging fluoroquinolone resistance in bacterial keratitis: a 5 year review. *Ophthalmology*, 1999; 106:1313-8.
6. Kowalski RP, Lisa MK, Romanwaski EG. Infectious disease: changing antibiotics susceptibility. *OphthalmolClin North Am* 2003; 16:1-9.
7. Alexandrakis , Eduardo CA, Darlene M. Shifting trends in bacterial keratitis in South Florida and emerging resistance to fluoroquinolones. *Ophthalmology* 2000; 107: 1497-1502.
8. Mathar R, Lisa MK, Romanowski EG, Kowalski RP. Fourth Generations Fluoroquinolones : New weapons in the Arsenal of Ophthalmic Antibiotics. *Am J Ophthalmol* 2002; 133: 463-466.
9. Darlene Miller et al: ocular flora & their antibiotic resistance patterns in the Midwest: A prospective study of patients under going cataract surgery *Am J Ophthalmology* 2013, 155:36-44.
10. Hass W et al Antimicrob agents chemothera 2009 Aug. 53(8): 3552-60 besifloxacin, a novel fluorquinolone has broad spectrum in vitro activity against aerobic and anaerobic bacteria
11. Penny A asbell et al, Antibiotic Resistance traends Among ocular pathogen in the US cumulative results from the antibiotic resistance monitoring in ocular micro organism (ARMOR) surveillance study 2015.