

TITLE
EMERGENCE OF RESISTANCE TO FLUOROQUINOLONES AMONG OCULAR PATHOGENS

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Abstract

Introduction: The fluoroquinolones are effective against host of Gram negative, Gram-positive microorganisms and anaerobes. Due to misuse, inappropriate dosing and schedule, Incidence of in vitro resistance to older Fluoroquinolone is increasing.

Methodology: 200 patients suspected of bacterial ocular infections were chosen for study. Bacterial Cultures were performed and sensitivity testing done by disc diffusion method. They were graded as 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: This prospective study shown that resistance against older fluoroquinolones is more in comparison to newer ones. This resistance can be reduced by proper use of antibiotics, culture and sensitivity study in serious infections and use of fluoroquinolones on the basis of susceptibility profiles, using mutants prevention concentration.

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I. Introduction

External ocular surface contains bacteria as microbial flora. several protective mechanisms operate on the eye surface which prevent eye infections. Breach in surface epithelium predispose the eye to bacterial infections 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 are broad spectrum antibacterial agents, which are effective against a host of Gram negative, Gram-positive microorganisms and anaerobes. But due to misuse, inappropriate dosing and schedule, in vitro resistance to older Fluoroquinolone is increasing which 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 older ones. Fluoroquinolone inhibit DNA gyrase and Topoisomerase IV.^{1,2}

Fluoroquinolone resistance develop through 2 main mechanisms-

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

II. Material And Method

This was a Prospective study, done on 200 outdoor and Indoor patients in department of ophthalmology, government medical college, Pali from June, 2019 to June, 2020.

INCLUSION CRITERIA

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

EXCLUSION CRITERIA

Fungal corneal ulcer, Viral corneal ulcer, Any preexisting septic foci, any viral/ fungal/ protozoal ocular infection. Informed consent was taken from all subjects and cultures were performed. Fluoroquinolone Susceptibility testing of each isolate was assessed clinically and in laboratory.

In vitro susceptibility testing was determined by Disc Diffusion method (KIRBY –BAUER)⁴ 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 inoculums of strictly standardized density on Muller Hinton Agar. Sensitivity was graded as

sensitive,intermediate,resistant by comparing the diameters of inhibition zone with critical zone diameter according to CLSI guidelines.

III. 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	50	25 %
2	21-40	82	41%
3	41-60	46	23%
4	>60	22	11%

Table -2 Sex Ratio Distribution

Disease Group	Male		Female	
	N	%	N	%
Disease of Lacrimal Apparatus (Acute Dacryocystitis, Lacrimal Abscess)	5	2.5%	18	9%
Disease of Conjunctiva (Acute Conjunctivitis)	26	13%	17	8.5%
Disease of Cornea (Keratitis)	67	33.5%	34	17%
Miscellaneous (Stye, ulcerative blepharitis, perforating injury)	18	9%	15	7.5%
Total	116	58%	84	42%

Table -3 Demographic Variation

Disease Group	Rural		Urban	
	N	%	N	%
Disease of Lacrimal Apparatus (Acute Dacryocystitis Lacrimal Abscess)	4	2%	19	9.5%
Disease of Conjunctiva (Acute Conjunctivitis)	25	12.5%	18	9%
Disease of Cornea (Keratitis)	68	34%	33	16.5%
Miscellaneous (Stye, Ulcerative Blepharitis, Perforating injury)	20	10%	13	6.5%

Table -4 Distribution of Bacterial Isolates Identified

Bacteria	Total	Percentage
Gram -Positive Bacteria	142	71%
Gram-Negative Bacteria	58	29%
Total	100	100%

TABLE 5 SENSITIVITY AND RESISTANCE OF GRAM -POSITIVE BACTERIA TO FLUOROQUINOLONES (TOTAL NUMBER OF CASES, N-148)

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	76	53.52	66	46.47
2	Ofloxacin	84	59.15	58	40.84
3	Gatifloxacin	110	77.46	32	22.53
4	Moxifloxacin	113	79.57	29	20.42
5	Besifloxacin	142	100	0	00

TABLE 6 SENSITIVITY AND RESISTANCE OF GRAM -NEGATIVE BACTERIA TO FLUOROQUINOLONES (TOTAL NUMBER OF CASES,N-52)

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

		N	Percentage	N	Percentage
1	Ciprofloxacin	42	72.41	16	27.58
2	Ofloxacin	44	75.86	14	24.13
3	Gatifloxacin	46	79.31	12	20.68
4	Moxifloxacin	49	84.48	9	15.51
5	Besifloxacin	58	100	00	00

Age of patients range from 5 days up to 73 years (Table No-1) in our study. The maximum number of patients were in the age group of 21 to 40 years ,i.e. 82(41%) out of total 200 patient .Due to much more involvement in outdoor activities, younger age group are more prone to trauma and excessive exposure to environmental risk factors, i.e.dry heat, dust, sunlight etc., so higher incidence of bacterial infection and longer duration of time is reported in them in comparison to older age group.

Due to more outdoor activities of males in comparison to females, we found in our study (Table No-2) that males are slightly more involved 116(58%) as compared to84(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. (Table No 3) shows that incidence of ocular infection in rural population 58.5% was more as compared to Urban population 41.5%

In our research work as shown (Table No- 4) out of 200 in vitro - culture identified, 142 were Gram Positive Bacteria while 58 were Gram Negative bacterial isolates. The ratio of Gram Positive Bacteria to Gram Negative bacteria comes out to be 2.44 : 1

.Similar results are seen in a 5 years retrospective review study conducted by Goldstein et al⁵ 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 ⁶, Aleandrakes et al ⁷.

(TABLE No- 5) shows the comparison between all 5 fluoroquinolones. Among Gram Positive Bacteria, resistance to ciprofloxacin shown in 66 (46.47%) cases, ofloxacin in 58(40.84%) cases, gatifloxacin in 32 (22.53%)cases, moxifloxacin in 29(20.42%)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 16 cases(27.58%), ofloxacin in 14(24.13%) cases ,gatifloxacin in 12 (20.68%)cases, moxifloxacin in 9(15.51%)cases, besifloxacin in zero case. This shows that no significant emerging resistance is there for newer as well as older fluoroquinolones on the basis of laboratory and clinical evaluation .It means that older fluoroquinolones are still promising for the treatment of gram negative bacterial ocular infections. Emerging resistance among ocular pathogens mainly Gram positive organisms to older fluoroquinolones, 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 piperazinyl ring.Besifloxacin, a C8-chloro-fluoroquinolone newer fluoroquinolone.

Mathar et al⁸ 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 al⁹ 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 al¹⁰ 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 al¹¹ 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.

IV. Conclusion

1. Our clinical study of ocular pathogens shows that there is emergence of resistance to older fluoroquinolones like Ciprofloxacin and Ofloxacin in ocular pathogens which is mainly Gram positive organisms.
2. 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 mutant prevention concentration and modifying the dose regimens.
3. The resistance against the newer fluoroquinolones is less in comparison to older fluoroquinolones

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.

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