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Research Article

Conjunctival bacterial isolates and their susceptibility Pattern in patients undergoing Cataract surgery at Khartoum Tertiary Eye Hospital, Khartoum-Sudan.

Mohammed Abdelgadir Abdelmahmoud[1] and Wafa Ibrahim Elhag[2]

[1]Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum-Sudan.

P.O. Box 12702, Postal Code 11121-Khartoum.

[2]Medical Microbiology Department, Deputy Dean, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum-Sudan.

ABSTRACT

Background: Post-operative infection following Cataract surgery was increased in recent years; moreover the causative agents have increased drugs resistance. The specific objective of this study was to identify conjunctival bacterial isolates and to determine their antimicrobials susceptibility pattern in pre-cataract surgery patients during the study period. **Methods:** This cross sectional study involved 66 pre-Cataract surgery patients who were potentially eligible to our study. The study was conducted from December 2015 to April 2016 in the department of Ophthalmology at Khartoum Tertiary Eye Hospital. Ocular swabs pre-moisture with normal saline were collected from both conjunctivas. Isolation and identification of bacteria was performed using conventional microbiological methods. Antimicrobials sensitivity test was done using standard disk diffusion method. **Results:** 79 bacterial strains were recovered from both conjunctivas. Gram positive microorganisms were 92.4% isolates, of which *B. cereus* 34.2%, *E.faecalis* 24% and *CoNS* 22.8% were the most common frequent isolates respectively. Whereas Gram negative microorganisms isolated from 7.6% of cases of which *M.catarrhalis* 3.8%, *Enterobacter* spp 2.5% and *K.pneumoniae* 1.3% respectively. Antimicrobials susceptibility test showed, low resistance rates to Cefotaxime 16.7%, Chloramphenicol 23.3%, Vancomycin 26%, and Amikacin (33.3%) respectively. From the total bacterial isolates 44.3% o*f which, Enterococcus faecalis* 15.2%, *Bacillus cereus* 13.9%, *CoNS* 10.1%, *Enterobacter* spp 2.5%, *St.viridans* 1.3% and *Micrococus* spp 1.3% showed multidrug resistance (MDR).

Conclusion: The most common bacterial isolates found on conjunctival swabs of pre-cataract surgery patients at Khartoum Tertiary Eye Hospital were, *B.cereus, E.faecalis, CoNS, M. catarrhalis, Enterobacter* spp and *K.pnumoniae*. Cefotaxime, Chloramphenicol, Vancomycin and Amikacin showed lowest resistance rates to all bacterial isolates.

Key words: Bacterial isolates, conjunctiva, Antimicrobials, pre-cataract surgery, Khartoum-Sudan.

INTRODUCTION

Bacteria are major cause of ocular infections and possible loss of vision, also the emergence of antimicrobial resistance bacteria increase the risk of treatment failure with potentially serious consequences[1]. Endophthalmitis is an inflammatory condition of the eye often caused by bacterial infection, it is rare but dreaded complication of cataract surgery, as it leads to high ocular morbidity

and visual loss even with antibiotic treatment[2]. Most bacteria responsible for post-operative ocular infection are part of the normal microbial flora of the conjunctiva and eyelids of the patients. Also, instruments or intraocular lenses may become contaminated if they touch the ocular surface[2]. Gram positive organisms are identified in about seventy percent of culture proved cases of Endophthalmitis[3]. Studies suggested that *Staphylococcus epidermidis* was the most commonly isolated organism, responsible for about forty percent of cases. *Staphylococcus aureus* is identified in approximately twenty percent of cases, while Gram negative organisms are implicated in sixteen percen[3]. DNA studies in *Staphylococcus epidermidis* endophthalmitis suggested that the commonest source of infection was the patient's own flora, organisms may be carried into the eye as surface fluid refluxes through the wound during surgery[3]. Despite this evidence it is accepted that routine pre-operative conjunctival swabs do not have a role in pre-operative preparation. Inconstantly, in the same studies there was little in cases of delayed acute post-operative endophthalmitis, microorganisms be gained from conjunctival swabs, pre-operatively[3]. There appears to be variability in the isolation of potential pathogens using daily swabs such as *Staphylococcus aureus* was isolated from over twenty percent of lid margin samples despite previously negative culture, however the act of taking a swab itself modify lid flora[3]. The type and pattern of organisms that cause ocular infections changes overtime. Moreover, the causative agents have developed increased drug resistance[4].In culture dependent studies, over fifty percent of swabs from the conjunctiva showed growth of skin-like bacteria mostly *coagulase-negative staphylococci*, *Propionibacterium*, and *Corynebacterium*[5]. Consistently, evidence suggested that transplantation of microbiota between body sites changes bacterial population structure only temporarily, as in other body sites, the ocular microbiota is expected to play a defensive role against colonization of pathogens in the eye[5]. Previous studies have suggested that the commensal of microbiota of ocular surface could interact with the host in immune system to suppress microbial pathogenicity, despite being important in ophthalmology, the eye microbiome has been largely neglected, and its functions remain unknown[5]. *Bacillus cereus* and other *Bacillus* spp, a group of Gram positive spore forming bacilli were the most frequently isolated organisms from Endophthalmitis resulting from traumatic injury[6]. The onset of symptoms is sudden, and the course is fulminant, release of necrotizing enzymes can result in loss of the eye within 48 hours. However, *B. cereus* eye infections were the most common type of non-gastrointestinal infection caused by this organism; these include Endophthalmitis, panophthalmitis and keratitis with abscess formation[6]. Use of prophylactic antibiotics in cataract surgery reduce the number of organisms in the conjunctiva and eyelids and thus reduce the risk of post-operative infection[7].Trends of bacterial resistance have been shown to increase among commonly used antibiotics, however the trend is variable to topical flouroquinolones, a group of broad spectrum bactericidal agent most frequently used as pre-and post-operative prophylaxis for ocular surgeries[7]. In recent years there has been significant public and professional concern regarding the use of chloramphenicol eye drops because of the associated risk of bone marrow Aplasia[7]. Furthermore, there was no enough relevant data in Sudan. This study aimed to identify Conjunctival bacterial isolates and to determine their Antimicrobials susceptibility pattern in pre-cataract patients submitted to surgery.

METHODS

Study design and sampling process:

This descriptive cross-sectional study design was conducted from December 2015 to April 2016 in the Department of Ophthalmology at Khartoum Tertiary Eye Hospital-Central Khartoum, Khartoum-Sudan. The study involved sixty six pre-cataract surgery patients more than eighteen years old, any subject who doesn't mate inclusion criteria were excluded from study, (Figure 1).

Study clearance and ethical consideration:

A permission to conduct this study was obtained from Al Neelain Ethical Committee with collaboration of Ministry of Health, Khartoum State-Sudan. A written informed consent form was obtained from study Participants confidentiality after they given them anonymous codes.

Data collection and Laboratory procedures

Demographic data was obtained using specified instructed standardized-interviewer administered face to face questionnaire. Regression analysis that revealed combination of history and clinical signs and symptoms has had been done by professional ophthalmologist using calibrated slit lamp bio-microscopy.

Collection of ocular swabs:

Dry sterile cotton swabs (GM Lab®, China) pre-moisture with normal saline 0.85% were used to swab sampling sites. Sites sampled included right and left conjunctivas. Samples were collected aseptically

from study participants without touching eyelids and eye lashes and before the application of the anesthetic, povidone iodine and the dilated eye drops. Samples were collected in the operation room under aseptic condition and then inoculated immediately aseptically into enriched medium (brain heart infusion broth with cooked meat particle (BHI-CMP) (Hi media®, India), to support the growth of aerobes and facultative anaerobic microorganisms, samples incubated overnight in ambient air at room temperature and then proceeded to reference microbiological laboratory using a standard operating procedures (SOPs) in the department of microbiology at the faculty of Veterinary Medicine, University of Khartoum, Khartoum North, Shambat-Sudan.

Culture and identification:

Ocular swabs incubated overnight in brain heart infusion broth with cooked meat particles (BHI-CMP) (Hi Media®, India) , showed significant growth and then were plated into 5% sheep blood agar (SBA), chocolate agar, aerobically and anaerobically, with addition of 5-10% Co_2 , MacConkey agar (Hi Media®, India) aerobically ,at 37°C for 24-72 hours of incubation.

Identification of isolated bacteria was based on conventional microbiological methods, these included for Gram positive bacteria, indirect Gram stain, hemolytic activity on 5% sheep blood agar (SBA), 3% catalase reaction test , coagulase reaction (tube method), optochin disk test ,bacitracin disk test, 0.04% pencillin G disk test, novobiocin disk test, motility test, CAMP test,1% oxidase test, 40% Bile esculin hydrolysis, growth on NaCl 6.5%, and sets of sugars such as 1% of Manitol, Arginine, Raffinose Pyruvate, Arabinose, and Sorbose. For Gram negative bacteria identification was based on indirect Gram stain, colony morphology on blood agar, MacConkey agar, and reaction on triple sugar iron, hydrogen sulfide production, Indole test, motility test, citrate test, urease test, and oxidase test.[8,9,10].

Antimicrobials susceptibility pattern:

Antibiotic multi disks (Hi Media®, India) matching Clinical Laboratory Standard Institute (CLSI) breakpoints were performed to clinically isolated bacteria using a standard disk diffusion method for drug susceptibility test (DST), and were done on Muller Hinton Agar (Hi Media®, India), with 0.5 McFarland turbidity standards .The following antibiotic disks which available with varying concentrations were used for testing, Chloramphenicol (C) 30 mcg, Ceftriaxzone (CRX) 30 mcg, Vancomycin (VA) 30 mcg, Erythromycin (E) 15 mcg, Tetracycline (TE) 30 mcg, and Clindamycin (CD) 2 mcg (Hi Media®, India) for gram positive bacterial isolates. For gram negative bacterial isolates we used, Cefotaxime (CXT) 30 mcg, Amikacin (AK) 30 mcg, Tetracycline (TE) 30 mcg, Co-trimaxzole (C), 15 mcg , Ampicillin (AMP) 10 mcg, and Cefuroxime (CXM) 30 mcg (Hi Media®, India).The Zone size Interpretative chart was based on results obtained using Mueller Hinton Agar[11]. Multi drug resistance (MDR) bacteria were defined as isolates which are resistance to three or more classes of drugs[12].

Quality Control (QC):

Aseptic techniques were strictly observed during samples collection, transportation, processing and storage. All reagents and kits were used according to manufacturer instruction sheet. Biological materials used include 5% sheep blood, rabbit de-fibrigenated plasma, standard strains for quality control, *Staph aureus* ATCC 25923, *Staph aureus* ATCC 43300, *Staph epidermidis* ATCC 12228, *Escherchia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Statistical analysis:

Data analysis was performed using SPSS version 21 according to study objectives. Descriptive data analysis (Frequency and percentage) on subject demographics were calculated using Mean, SD[13-15].

Patient Clinical data and frequency of Cataract surgery out-patients and operations (phacoemulsification and intraocular lens implantation) among males and females was obtained from hospital medical records during study periods (Figures 2. 3 .4).

RESULTS

Socio-demographic characteristics of the study participants:

Out of 66 pre-cataract surgery patients recruited in the study, the mean age was 60 years old (range 45-75 years) ,with an ideal weight ,Body Mass Index (BMI)=21.9 Normal (18.0-24.9),a number of (34/66,51.4%) were males. The majority of study participants (47/66, 71.2%) were lived in rural areas, with low educational level.

Conjunctival bacterial isolates:

A total of 79 bacterial strains were isolated from both conjunctivas of studied group, (73/79, 92.4%) of the isolates were belonging to Gram-positive bacteria, with higher abundances of *Bacillus cereus* (27/79, 34.2%), followed by *Enterococcus faecalis* (19/79, 24%), and *Coagulase negative Staphylococci* (CoNS) (18/79, 22.8%), while Gram negative bacteria were

(6/79, 7.6%), of which *Moraxella catarrhalis* (3/79, 3.8%), followed by *Enterobacter spp* (2/79, 2.5%), and *Klebsiella pneumoniae* (1/79, 1.3%), were the most common frequent isolates respectively, (Figure 5).

Susceptibility of bacterial isolates to antibiotics:

Regarding drug susceptibility pattern of bacterial isolates, a number of 79 bacterial strains were tested for drugs susceptibility pattern, the result showed highest resistance rates of Gram-positive bacteria occurred against Erythromycin (49/73,67.1%), Clindamycin (46/7,63%),however Chloramphenicol showed the lowest resistance rates (17/73,23.3%), followed by Vancomycin (19/73,26%), Tetracycline (23/73,31.5%), and Ceftriaxzone (26/73,35.6%) respectively (Figure 6).

For Gram negative bacteria, it showed highest resistance rates against both Cefuroxime, Ampicillin 4/6(66.7%), whereas, lowest resistance rates against Cefotaxime (1/6, 16.7%), followed by Amikacin (2/6, 33.3%) respectively, (Figure 7).

Multidrug resistance isolates (MDR) (isolates showed resistance to three or more groups of antibiotics), a number of thirty five isolates (35/79,44.3%), *E.faecalis* (12/79,15.2%), B.cereus (11/79,13.9%), *Coagulase Negative staphylococci* (CoNS) (8/79,10.1%), *Enterobacter spp* (2/79,2.5%), St.viridans (1/79,1,3%), and *Micrococcus spp* 1/79(1.3%) showed multidrug resistance, (Figure 8).

DISCUSSION

The organisms that cause ocular infection are generally exogenous. However, in certain circumstances they gain access to enter the eye and cause infection[1].

In the present study different pathogens were isolated from conjunctiva of pre-cataract patients prior to surgery. Gram-positive bacteria were commonly microorganisms being isolated, with high prevalence rates (92.4%), this finding was similar to closely related study[2], which showed that gram positive microorganisms were 86%. Also, Ansari MR, Madani H, Ghaderi E, reported that bacteria were the most common group of causative agents of endophthalamitis, and gram positive pathogens were responsible for about 60 to 80% of acute infections[16].

In our study, *Bacillus cereus* being the most common isolate (34.2%), followed by *Enterococcus faecalis*(24%), and *Coagulase negative staphylococci* (CoNS) (22.8 %), this finding was different from other similar studies which showed that *Coagulase negative staphylococci* (CoNS), were the most common frequent isolates, followed by *Staphylococcus aureus* and *streptococcus* spp[1,2,4,7,12] this may be attributed to different strains of isolated pathogens and may to different study regions, such as *B. cereus* which was inoculated to the eye with contaminated dust or dirt particles. *Enterococcus faecalis* which was found as natural inhabitant of the gastro intestinal tract of human and animals, but it can be found in our current study, constantly, in other study which was done in South Korea and Sweden, showed that *Enterococcus* species (spp) have recently emerged as a leading cause of endophthalmitis[17]. The authors found that *Enterococcus faecalis* was the most common causative organism, accounting for 28.4% of all culture positive post-operative cases of endophthalmitis[17].Additionally, in other a prospective study, done by friling et al on Swedish patients found that *Enterococcus* spp were the leading cause of post-operative endophthalmitis[18].

In contrast, Gram-negative bacteria were less than 10% of cases, this finding was consistent with other related studies[1,2,4], but it was different from another studies[7,12], which showed that Gram negative isolates were (20%, 25%) respectively.

The present study found high resistance rates of the Gram-positive isolates to Erythromycin (67.1%), and Clindamycin (63%), this finding was similar to other studies[1,2,4,12], this may be due to prolonged use of antibiotics and common practice of self-medication in Sudan. In this study, Chloramphenicol showed low resistance rates (23.3%), this finding was different from other related study[12], which showed high resistance rates to Chloramphenicol (52.7%).

In the present study, multidrug resistance (MDR) isolates were (44.3%) this finding was different from other closely related studies, which was done in Northwest Ethiopia and Egypt[7,12], showed that multidrug resistance isolates (MDR), were (87.1%, 97.2%), respectively.

CONCLUSION

The most commonly bacteria found on conjunctivas of the pre-operative cataract surgery patients at Khartoum Tertiary Eye Hospital are, *Bacillus cereus, Enterococcus faecalis, Coagulase negative staphylococci, and Moraxella catarrhalis.*

Majority of these isolates showed high resistance rates to Erythromycin, Clindamycin, Cefuroxime, and Ampicillin. In contrast, Cefotaxime, Chloramphenicol, Vancomycin, and Amikacin showed low resistance rates within antibiotics. Therefore, these antibiotics may be used to reduce post-operative infection following cataract surgery.

Accordingly, we recommend further more studies with larger sample size to clarify this association. Also, a high level of infection control precaution should be applied during cataract surgery. Great care

also, has to be taken for the choice of prophylactic antibiotics following Cataract surgery to prevent post-operative Endophthalmitis.

DECLARATIONS

Ethics approval and consent to participate

The study protocol was approved by Al Neelain Ethical Committee .Ethical clearance for clinical study was obtained from Ministry of Health, Khartoum State-Sudan, (approval letter reference No: 44/A) .We obtained the approval of patients for use of their clinical data with written informed consent form.

Consent for publication

Not applicable**.**

Availability of data and materials

All the data supporting our findings is contained within the manuscript.

Competing interests

The authors declare that they have no competing interests.

Authors' Contributions

MAA, ES participated in the design of the study and performed statistical analysis of Data .WIE, FG Conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript. MAA collected data of patients and WIE participated in critical review of article. MAA, WIE drafted manuscript and approved final version of manuscript.

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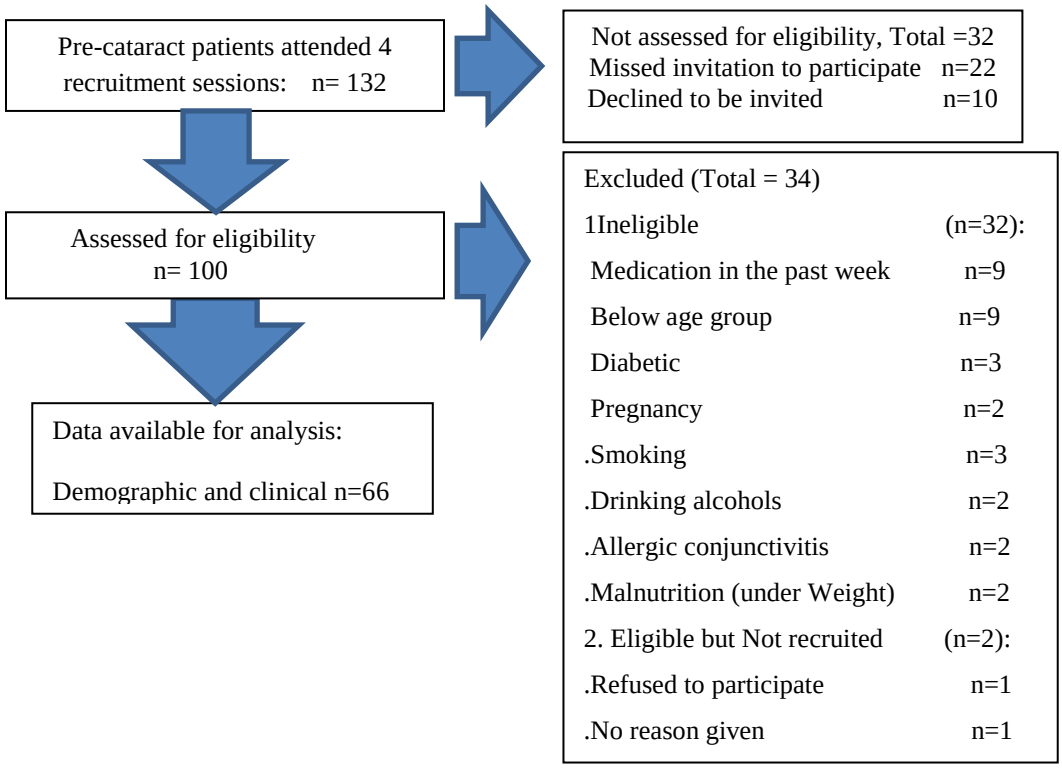

Fig 1
Eligibility criteria flow diagram.

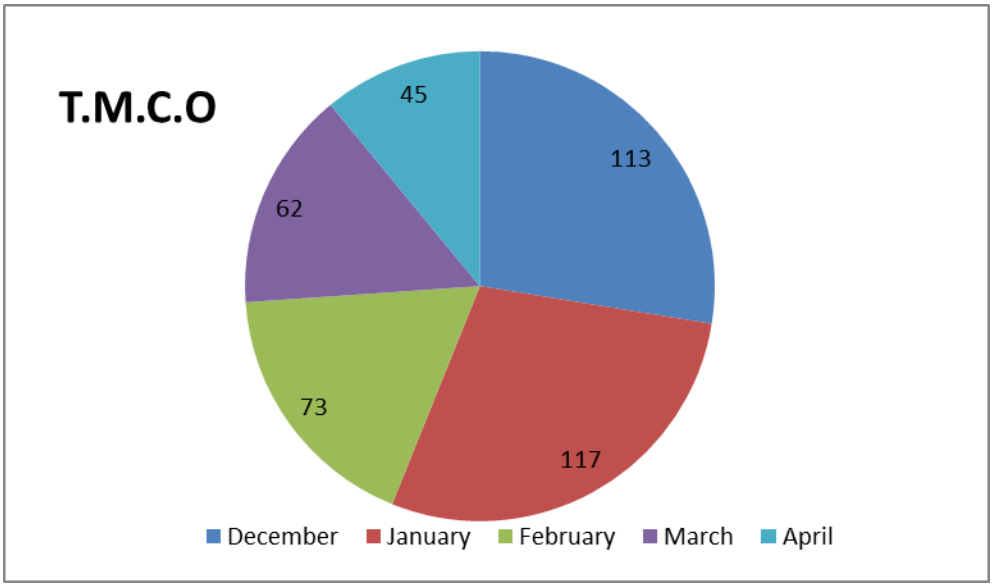

Fig 2
Frequency of Total Cataract Operations among males (T.M.C.O) during Study period.

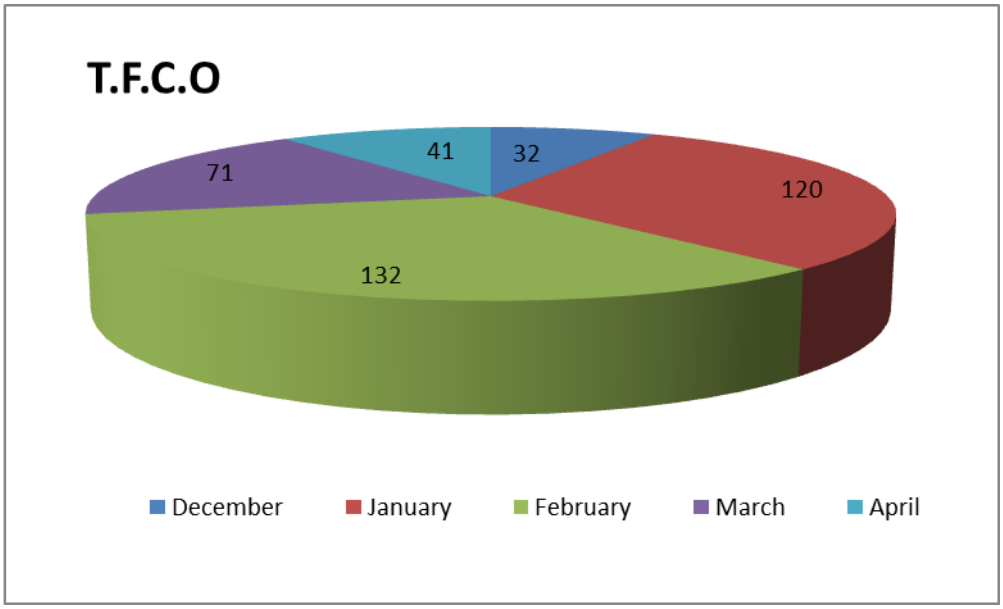

Fig 3
Frequency of Total Cataract Operations among females (T.F.C.O) during Study period.

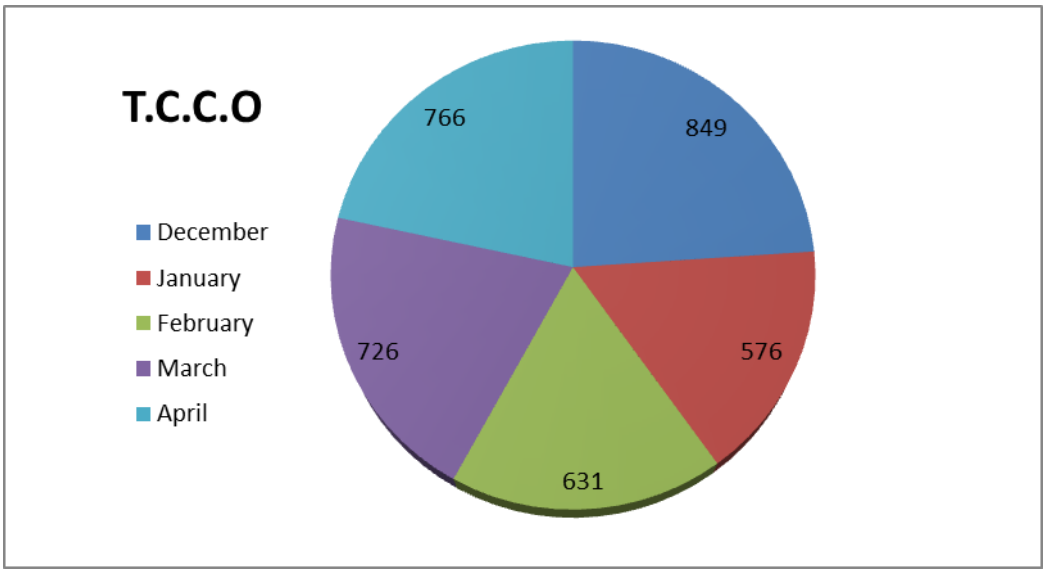

Fig4
Frequency of total Cataract clinic out- patients (T.C.C.O) during study period.

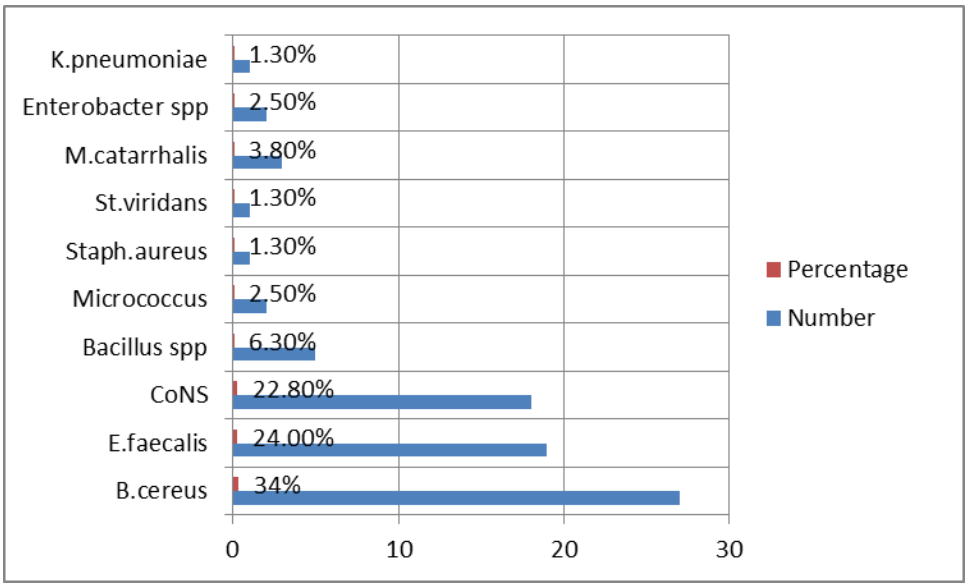

Fig 5
Frequency of different bacterial pathogens isolated from Conjunctival Swabs of pre-cataract surgery patients during study period (n=79).

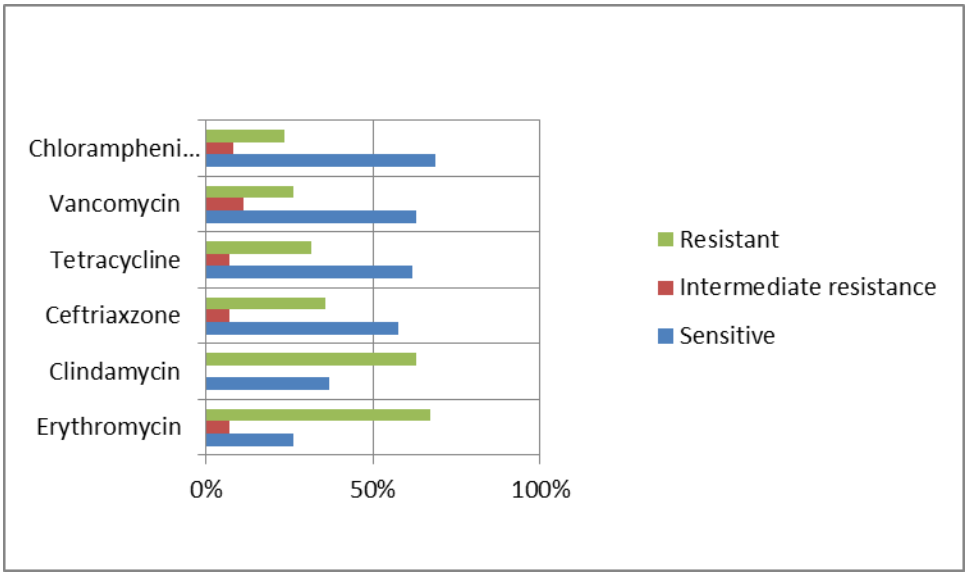

Fig 6
Frequency of Antimicrobials susceptibility pattern of Gram positive bacterial isolates (n=73).

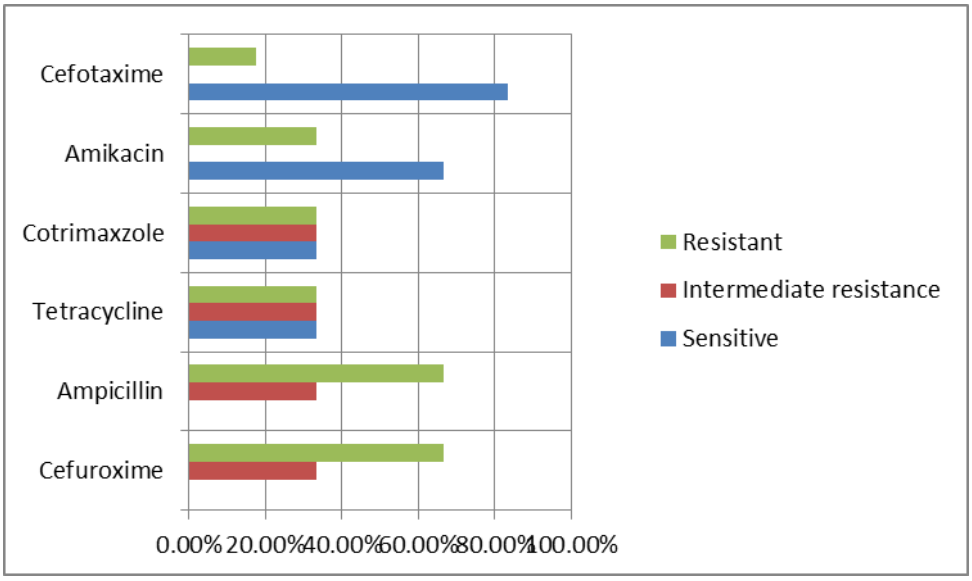

Fig 7
Frequency of Antimicrobials susceptibility pattern of Gram negative bacterial isolates (n=6).

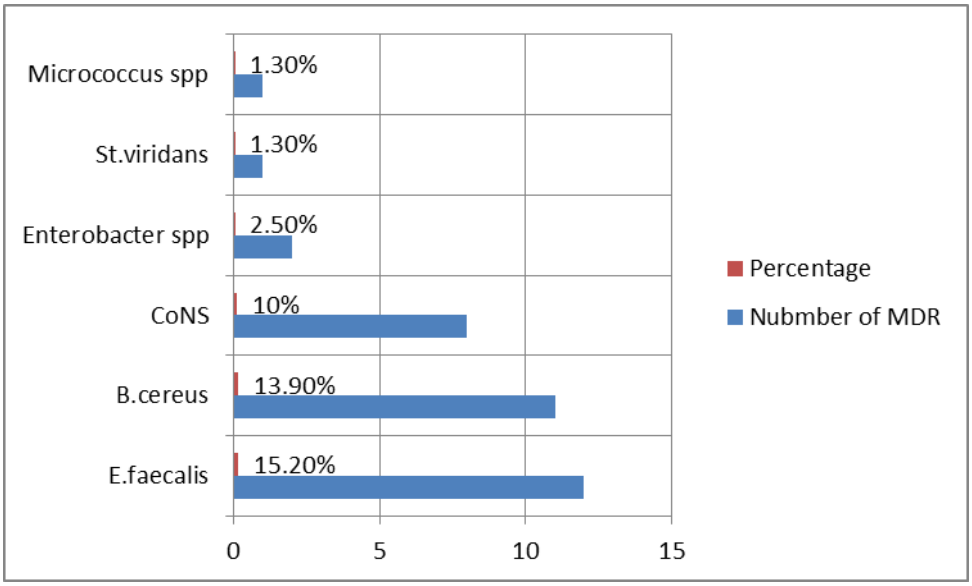

Fig 8
Frequency of Multi Drug Resistance bacteria (MDR) of 79 bacterial Isolates (n=35).

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