Isolation, Identification and Antimicrobial Susceptibility Patterns of Bacteria in Patients with Eye Infection in Khartoum State, Sudan

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Abstract:
   Bacteria are major cause of ocular infections and possible loss of vision. The emergence of antimicrobial resistant bacteria increases the risk of treatment failure with potentially serious consequences. The aim of this study was to determine the prevalence of bacterial isolates

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and their antimicrobial susceptibility pattern among patients with ocular infections. A cross-sectional study was conducted among 52 patients with external ocular infections at El-Waledain and Abd Elfadeil Almaz Specified Hospital, Khartoum. Socio-demographic and clinical data were collected using structured questionnaire. The specimens were collected by using sterile cotton swabs and then cultured in blood ager, chocolate agar and MacConkey agar, the identification of isolates done by several biochemical tests, Susceptibility pattern of isolated bacteria to antibiotics done by disc diffusion method. Out of fifty two eye swab specimens investigated, 22(42.3%) from males and 30(57.7%) from females. The overall prevalence of bacterial pathogens among samples were 47(90.4 %). Bacteriological tests showed that 25 (53.2%) were Gram positive and 22(46.8%) Gram negative bacteria. The frequencies of bacteria appear as; Staphylococcus epidermidis40.8% (n=22/47), Staphylococcus aureus 6.4% (n=3/47), Serratia marcescens 4.3% (n=2/47), and Pseudomonas aerugionsa was 42.6 % (n=20/47). From the above findings we concluded that, the prevalence of bacterial pathogens among eye swab samples was high and the predominant isolate were Staphylococcus epidermidis, Pseudomonas aerugionsa, Staphylococcus aureus and Serratia marcescens respectively. With regard to sensitivity to antibiotics six antibiotics were used; (Tetracycline, Ciprofloxacin, Gentamicin, Chloramphenicol, Tobramycin, Methicillin, Vancomycin, last two one for Staphylococcus aureus only), the result showed that the bacterial isolate were highly suspected to these antibiotics, Staphylococcus aureus strain collected were MRSA and Pseudomonas strain showed resistant to tetracycline which was previously administered as ointment or eye droplets. This study has shown the high percentage of eye infections among patients, so the study recommended that eye infections patients should be subjected to routine eye swab culture and determination of sensitivity to clinical isolates in order to select appropriate antimicrobial agents.

Key words: Isolation, Identification, Antimicrobial susceptibility patterns, Eye infection.

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INTRODUCTION:

Infections of the eye result from direct contact with a virus or from viremic spread, Conjunctivitis (pink eye) is a normal feature of many childhood infections and is a characteristic of infections caused by specific adenovirus serotypes, measles virus, and rubella virus. Kerato-conjunctivitis, caused by adenovirus, HSV, or VZV, involves the cornea and can cause severe damage. HSV disease can recur, causing scarring and blindness. Enterovirus 70 and Coxsackie A24 virus can cause an acute hemorrhagic conjunctivitis. Cataracts are classic features of babies born with congenital rubella syndrome. Chorio-retinitis is associated with CMV infection in newborns (congenital) as well as in immune-suppressed people (e.g., those with acquired immunodeficiency syndrome [AIDS]) [1]. Common organisms cause eye infections include: *Staphylococcus aureus*, *Haemophilus influenza*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Moraxella lacunata*, *Neisseria gonorrhea*, *Chlamydia trachomatis* and *Bacillus cereus* [1]. The main objectives of this study were to isolate the causative agents of bacterial eye infection, to identify the causative agents of bacterial eye infections and to determine the susceptibility of these causative agents to different antimicrobial agents.

MATERIALS AND METHODS:

Study design:
This is descriptive cross-sectional study.

Study area:
This study was conducted in two hospitals in Khartoum state these were El-Waledein hospital and Abd Elfadeil Almaz hospital. The specimens were collected in these hospitals but

the practical part was carried out at Medical microbiology laboratory of Sudan University of science and technology.

Study duration:
This study was done from March to June 2015.

Sample size:
Fifty two samples were collected.

Selection criteria:
The selection criteria depend on the hospital attendance (out patients) and patients responding to the questionnaire.

PROCESSING:

Collection of specimens:
The specimens were collected from patients who were previously diagnosed clinically to have allergic or bacterial conjunctivitis. All the patient's eyes were characterized by red, swelling and productive white to yellowish discharge, (most of them painful). The specimen were collected under observation of physician, using aseptic technique to clean the area around the eye, from non-washed eye, sterile cotton swab was impregnated with sterile normal saline. The specimens were collected from the corner of eye to influence trapping of the microorganisms by flushing mechanism of tears; two specimens were collected from each patient. All the samples were cultured immediately in three agar plate from first swab; Blood agar plate, Chocolate agar plate and MacConkey agar plate. The second swab was used to perform direct Gram's stain.

Culture:
The swabs firstly were cultured in Blood agar plate (BAP) then Chocolate agar plate (CHO) and then MacConkey agar plate

(MAC). These cultures were incubated at 37°c for 24 hours. At the end of incubation period colonial morphology were observed.

IDENTIFICATION:

Gram smear:

Dried smear were fixed and covered with crystal violet stain for 30-60 seconds, then the stain was washed off with clean water, all the water were tipped off, and the smear was covered with Lugol's iodine for 30-60 seconds, then washed off with clean water, and decolorized rapidly (few seconds) with acetone-alcohol, decolorizer was washed immediately with clean water. The smear was covered with safranin, stained for 2   minutes, washed off with clean water. The back of the slide were wiped, cleaned and place it to air-dry. Smears were examined microscopically, first with the 40 objective to check the staining and to see the distribution of material, and then examined with the oil immersion objective to report the bacteria and cells [2].

BIOCHEMICAL TEST:

Oxidase test:

A piece of filter paper were Placed in a clean Petri-dish and 2 or 3 drops freshly prepared oxidase reagent were added, using a piece of stick, colony of the test organism was removed and was smeared it on the filter paper and looked for the development of a blue-purple color within a few seconds. Result blue-purple color positive oxidase test (within 10 seconds) and negative oxidase test no blue-purple color (within 10 seconds) [2].

Sugars fermentation, H_2S and gas production:

This is a multi-tests carried out in Kligler Iron Agar (KIA) medium. The test was performed by inoculating KIA medium. A straight wire was used to inoculate KIA medium. First the butt

was stabbed and then the slope was streaked in a zig-zag pattern, after inoculation. KIA reactions are based on the fermentation of glucose or lactose and the production of hydrogen sulphide. A yellow butt (acid production) and red-pink slope indicate the fermentation of glucose only. The slope is pink-red due to no fermentation of lactose, air bubbles or crack of medium indicate gas production and blackening throughout the medium indicate hydrogen sulphide (H_2S) production[2].

Indole test:

The tested organism was inoculated in a bijou bottle containing 3 ml of sterile peptone water, then incubated at 35–37°C for up to 48 hours. Production of indole was tested by adding 0.5 ml of Kovac's reagent. Examined for a red color in the surface layer within 10 minutes. Red surface layer indicate positive indole test and no red surface layer indicate negative indole test [2].

Citrate utilization test:

The tested organism was inoculated in Simmon's citrate agar, incubated overnight at 37°C. Positive citrate test is indicated by blue to green color, no change in color indicate a negative citrate test [2].

Urease test:

The tested organism was inoculated in urea agar medium, incubated overnight at 37°C, red/purple color indicate a positive urease test while yellow color indicate a negative urease test [2].

Catalase test:

It's used to determine which genus of the Gram-positive bacteria were catalase positive and which one was catalase negative. With a sterilized wood stick small amount of isolated organism was picked up and put it inside tube contain hydrogen peroxide. The appearance of gas bubbles indicates

positive result; also determine whether any of the Gram-negative bacteria contained this enzyme [3].

DNA-ase test:

This test was used to help in the identification of *S. aureus* which produces deoxyribonucleic (DNA-ase) enzyme from other *Staphylococcus* spp. The organism was cultured on medium which contain DNA, after overnight incubation at 37°C the colony tested by flooding the plate with weak hydrochloric acid solution, positive result read as clear area around colonies [2].

Coagulase test:

The test was used to differentiate *Staphylococcus aureus* (coagulase positive) from coagulase-negative *Staphylococci*; *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Coagulase is an enzyme that causes a clot to form. When bacteria are incubated with plasma, using a sterile pipette, measure 0.1 ml of the broth culture, and transfer this inoculum to a tube of plasma, place inoculated plasma tubes in the 35°C incubator, then examined by held the tubes in a semi-horizontal position to see the clot formation [4].

Antimicrobial susceptibility testing:

This method was used to evaluate the effectiveness of antibiotics against specific bacteria by Kirby-Bauer method. Muller-Hinton agar was inoculated with a culture of a bacterial isolate. After inoculation, antibiotic disks were placed on the agar surface; Plates were incubated at 37°C. After overnight incubation the zone of inhibition were measure by using a ruler and compare the result with sensitivity chart to demonstrate this bacterial sensitive or resistant to antibiotic applied.

Quality control:

The standard strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus*) with ATCC (American type culture collection) No (27853, 25923) respectively was brought from National public health laboratory and sensitivity testing was performed on Muller-Hinton agar, in similar way (Disk diffusion method) and condition to our isolated to determine the validity of the selective antibiotics.

Ethical consideration:

All samples were taken from patients after agreement and fulfilling consent form.

DATA ANALYSIS:

Data were analyzed by statistical package for social sciences (SPSS), frequencies and percentages were used and then the data were presented in tables and figures.

RESULTS:

Total of fifty two eye swab specimens were collected from two hospitals El-waledain and Abd Elfadeil Almaz specified on ocular infection describe in table (1). The specimen collected from two gender males/ females which describe in table (2). The eye swab specimens were processed for isolation, identification and susceptibility testing. The distribution of cases among target population according to their residence as follows: Khartoum state, Aljazeera state, White Nile state and The Northern state percentage demonstrate in table (3). Out of fifty two eye swab specimens, 47 yielded bacterial growth, while only 5 failed to demonstrate any bacterial growth (no growth). Out of the 47 isolates twenty two were coagulase negative *Staphylococci* (*Staphylococcus epidermidis*), nineteen were

Pseudomonas aeruginosa, three were *Staphylococcus aureus* and two *Serratia marcescens*. The frequencies and percentage describe in table (4). The percentage of growth rate among bacterial isolates was 90.4% in figure (1). The antimicrobial pattern which were examined among bacterial isolates were; tetracycline (TE), Chloramphenicol (C), Ciprofloxacin (CIP), Gentamicin (G), Tobramycin (TOB), Norfloxacin (NX), Methicillin (METH) and Vancomycin (VAN) in figure (2, 3, 4, 5).

Table (1): Distribution of eye swab specimens according to hospitals

Hospital	Number	Percentage (%)
El-Waledain	28	53.8
Abd Elfadeil Almaz	24	46.2
Total	52	100

Table (2): Frequency and percentage of eye swab specimens according to Gender

Gender	Number	Percentage (%)
Male	22	42.3
Female	30	57.7
Total	52	100

Table (3): Distribution of eye swab specimens according to geographical area

State	Number	Percentage (%)
Khartoum state	45	86.5
Gezira state	3	5.8
White Nile state	3	5.8
The Northern state	1	1.9
Total	52	100

Table (4): Frequency and percentage of bacterial isolates among the specimens

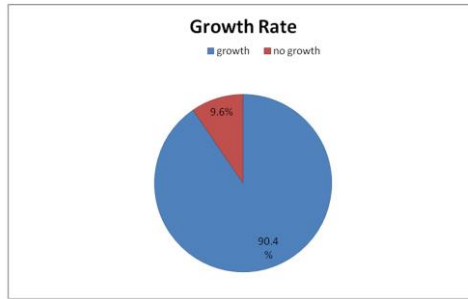| Isolate	Number	Percentage (%)
Staphylococcus epidermidis	22	46.81
Pseudomonas aeruginosa	20	42.55
S. aureus	3	6.38
Serratia marcescens	2	4.26
Total	47	100

Figure (1): The percentage of growth rate among bacterial isolates
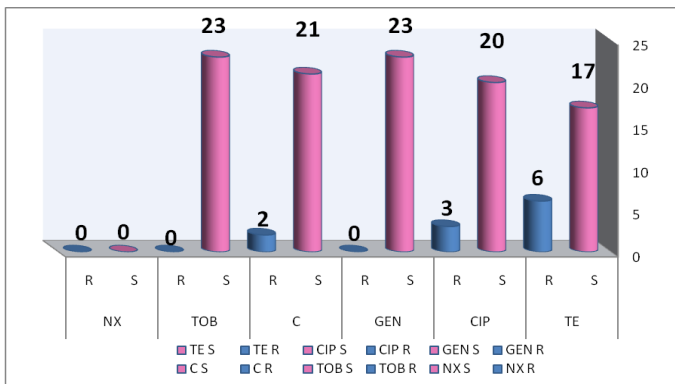

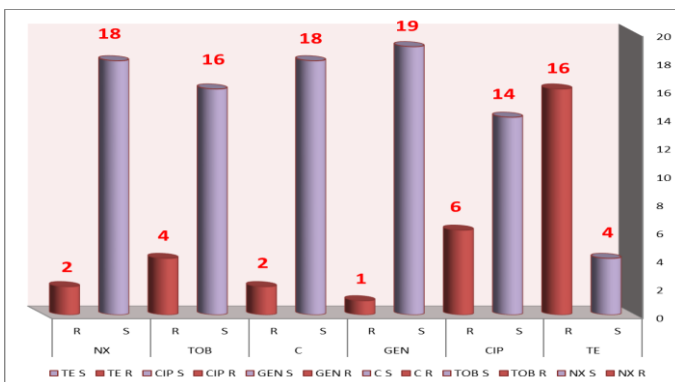**Figure (2): Susceptibility pattern of *Staphylococcus epidermidis* to antibiotics**

Figure (3): Susceptibility pattern of *Pseudomonas aeruginosa* to antibiotics

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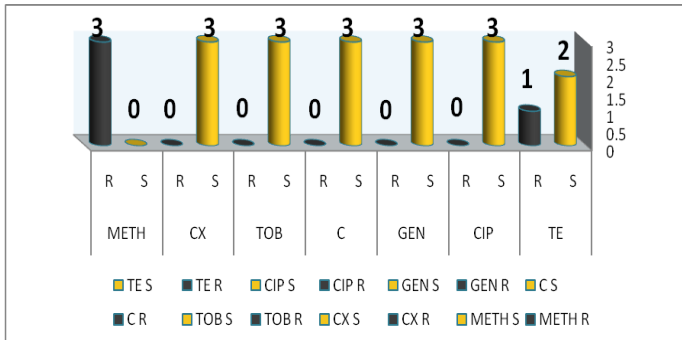

Figure (4): Susceptibility pattern of *Staphylococcus aureus* to antibiotics
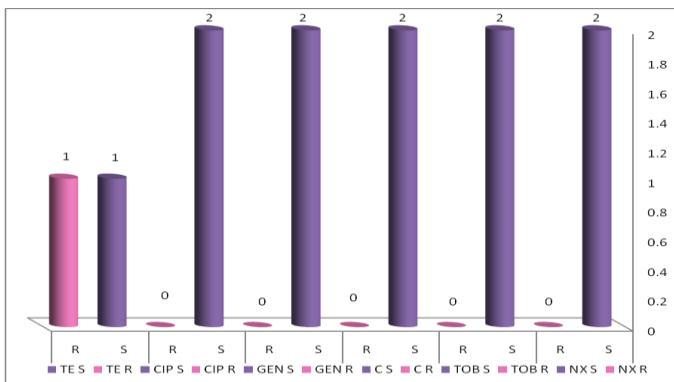

Figure (5): Susceptibility pattern of *Serratia marcescens* to antibiotics

DISCUSSION:

Fifty two patients suspected to have Ophthalmic infection were randomly selected for the present study, 47 (90.4%) of specimens show growth, while only 5 (9.6%) of specimens show no growth. 22 (42.3%) of them were males, 30 (57.7%) were females, with mean age (22 years). Equal percentage from two hospitals 26 (50%), the result were obtained from patients had no surgery except 4 (7.6%) of them, the study has revealed that the frequencies of isolated bacteria were 23 (44.2%) was

Staphylococcus epidermidis, 19 (36.5%) *Pseudomonas aeruginosa,* 3 (5.7%) *Staphylococcus aureus* and 2 (3.8%) *Serratia marcescens*. All Gram positive isolates were susceptible for vancomycin, but 100 % (2\2) *Staphylococcus aureus* were resistant to methicillin. Our results agree with the results obtained by Shiferawet *et al.* (2015) [5] in northeast Ethiopia who reported 59.4 %, the majority of the isolates (93.7 %; 89/95) were Gram positive and the other 6.3 % (6/95) Gram negative bacteria. The proportion of coagulase negative *Staphylococci* among the Gram positive bacterial isolates was 53.7 % (n= 51/95). All Gram positive isolates were susceptible for vancomycin but 67.4 % (n= 60/95) of them were resistant against amoxicillin. Also, agree with Reddy *et al.* (2010) [6] in south India results Out of 787 isolates, 147 (18.7%) were *Staphylococcus aureus*, 279 (35.2%) were coagulase-negative *Staphylococci*, other were streptococci species. Of the four quinolones, susceptibility to gatifloxacin was highest (85.6%) followed by ofloxacin (65.6%), moxifloxacin (63.9%), and ciprofloxacin (60.5%). Also, agree with Amir *et al.,* (2013) [7]. Bacterial conjunctivitis is the second most common cause of infectious conjunctivitis, with most uncomplicated cases resolving in 1 to 2 weeks. Lastly not finally our result were agree with Hemavathi *et al.* (2014) [8] in India where out of 235 specimens processed, 113(48%) showed growth. Conjunctival swabs yielded 39 (52%) bacterial isolates, predominant bacterial isolates were *Staphylococcus* species 36 (39.9%), *Pseudomonas* species 20 (22.2%) and *Escherichia coli* 12 (13.3%). Bacterial strains were susceptible to gatifloxacin (86.4%), tetracycline (65.4%), and chloramphenicol (69.1%) and least sensitive to the beta- lactam group like amoxicillin (23.5%). Final agreement with study of Khauv *et al.,* (2014) [9] in Cambodia where forty two patients (77.8%) were classified as having an external eye infection, ten (18.5%) as ophthalmia neonatorum and two (3.7%) asintra-ocular infection. Most

commonly isolated bacteria were *Staphylococcus aureus* (23 isolates), coagulase-negative *staphylococci* (13), *coli*-forms (7), *Haemophilus influenza /parainfluenzae* (6), *Streptococcus pneumoniae* (4) and *Neisseria gonorrhoeae* (2).

CONCLUSION:

This study concluded that the prevalence of bacterial pathogens among eye swab samples were high and the predominant isolate were *Staphylococcus epidermidis*, *Pseudomonas aerugionsa, Staphylococcus aureus* and *Serratia marcescens* respectively. Most effective antibiotics used as treatment which are broad spectrums for eradicate eye infection. Most strains of *S. aureus* isolated were methicillin resistant and sensitive to vancomycin.

REFERENCES:

1. Murray, R. P., Rosenthal, S. K. and Pfaller, A. M. *Pseudomonas* and related bacteria. Medical Microbiology. 7th edition. Philadelphia. 2013; 167-171, 288-296.
2. Cheesbrough, M. District Laboratory Practice in Tropical Countries. Part 2. 2nd edition. Cairo. 2004; 66-67.
3. Pollack, R. A., Findly, L., Monschein, W. and Modestro, R. R. (2009). Laboratory Exercise in microbiology. 3rd edition. USA. 2009; 169.
4. Harvey, R. A., Cornelissen, C. N. and Fisher, B. D. Lippincott's Illustrate Reviews 3rd edition. China. 2013; 24.
5. Shiferaw, B., Gelaw, B., Assefa, A., Assefa, Y. and Addis, Z. Bacterial isolates and their antimicrobial susceptibility pattern among patients with external ocular infections at Borumeda hospital in Northeast Ethiopia. *Bio Med Central ophthalmology*. 2015; **15**:103.

6. Reddy, A. K., Garg, P., Alam, M. R., Gopinathan, U., Sharma, S. and Krishnaiah, S. Comparison vitro susceptibilities of Gram-positive cocci isolated from ocular infections against the second and fourth generation quinolones at a tertiary eye care centre. *Eye South India.* 2010; **24**:170-174.

7. Amir, A. A. and Neal, P. B. Conjunctivitis: A Systematic Review of Diagnosis and Treatment. *National Institutes of Health.* 2013; **310**(16):1721-1729.

8. Hemavathi, S. P. and Shenoy, P. Profile of Microbial Isolates in Ophthalmic Infections and Antibiotic Susceptibility of the Bacterial Isolates A Study in an Eye Care Hospital, Bangalore. *Journal of Clinical and Diagnostic Research.* 2014; **8**(1):23-25.

9. Khauv, P., Turner, P., Soeng, C., Soeng, S., Moore, C. and Bousfield, R. Ophthalmic infections in children presenting to Angkor Hospital for Children. *Bio Med Central*, 2014; **784**:7.