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Research Paper

Evaluation of Bacterial Causative Agent of Conjunctivitis in Khartoum, Sudan

Microbiology

KEYWORDS : Conjunctivitis, Antimicrobial, Staphylococcus spp., Chloramphenicol, Neisseria gonorrhea, Agar.

Yagoub, Hamadt Allah Elhaj Abd Elseed	Assistant Professor in medical microbiology. Department of medical microbiology, Faculty of medical laboratory sciences. Um Dorman Islamic university (Sudan). Now: In department of Laboratory Medicine. Faculty of Applied Medical Sciences. Al -Baha University
Mohamed, A.EM. Ibrahim	Assistant Professor in Public Health. Department of Environmental Health, Faculty of public and Environmental Health. University of Khartoum (Sudan). Now: at Al -BahP, University (Saudi Arabia), Faculty of Applied Medical Sciences. Mobile:+966506384654U, E.mail: mohammedawad11@gmail.com
Amani Rahamt Allah Osman	B.Sc in Medical Microbiology. Department of Medical Microbiology. Faculty of Medical Laboratory Sciences. Um Dorman Islamic University, (Sudan).
Duaa Monerr Mahmood Elmahbob	B.Sc in Medical Microbiology. Department of Medical Microbiology. Faculty of Medical Laboratory Sciences. Um Dorman Islamic University, (Sudan).
Ghozaia Abdelraheem Mohammed	B.Sc in Medical Microbiology. Department of Medical Microbiology. Faculty of Medical Laboratory Sciences. Um Dorman Islamic University, (Sudan).

ABSTRACT

Background: Conjunctivitis is one of the most frequently occurring hospital-acquired infections, although it is less studied than potentially life-threatening infections, such as sepsis and pneumonia.

Objective: The aim of this study to isolate and identify the common bacterial causative agents of conjunctivitis and to determine the susceptibility of microorganism to common used antibiotics. Methods: A cross sectional study conducted in Abdalfadeel ALmath National Ophthalmic Center – Khartoum (Sudan). Fifty swabs were collected from patients attended to Abdalfadeel ALmath (Khartoum–Sudan) National Ophthalmic Center from March to July 2011. All specimens were cultivated in blood agar and chocolate agar, and then subjected to bacteriological identification and sensitivity tests.

Antimicrobial sensitivity tests were performed by disc diffusion method. Five types of antimicrobial were used; Chloramphenicol, Ciprofloxacin, Tobramycin, Tetracycline and Fusidic acid. Results: Out of fifty specimens forty five (90%) specimens showed positive growth, whereas five (10%) specimens showed negative growth. Forty (88.9%) from positive growth specimens were appeared Staphylococcus spp and five (11.1%) specimens were appeared Neisseria gonorrhea.

All isolated bacteria showed sensitivity to Chloramphenicol, Ciprofloxacin and Tobramycin, where as they showed resistance to Tetracycline and Fusidic acid (4%,14%) respectively. Conclusion: This study indicated that the bacterial conjunctivitis was commonly caused by staphylococcus species; also Neisseria gonorrhea was isolated from infant of mother having gonorrhea. Sensitivity tests indicated that Chloramphenicol, Ciprofloxacin, and Tobramycin were more effective than other antibiotics used against isolates bacteria identified in this study.

Introduction:

Eye is most sensitive organ; we observed that many patients start therapy. empirically without culturing the conjunctiva. The massive usage of broad-spectrum antibiotics increase resistance of organism, so culturing he conjunctiva before starting therapy may be warranted. Use of topical antibiotics ort orticosteroid antibiotics can induce toxicity and prolong viral infection, so we mustc. differentiate between bacterial and viral conjunctivitis Conjunctivitis spreads easily in poor hygiene and crowded areas such as schools, households nd hospitals. Therefore screening and awareness about etiological agents is essential toa. minimize transmission of disease The study aimed to achieve the following objectives, to isolate and identify the common bacterial ausative agents of conjunctivitis and to determine the susceptibility of microorganism to commonc. antibiotics used in Sudan

The conjunctiva is a thin, translucent, relatively elasti tissue layer with both bulbar and palpebral portions. The bulbar portion of the conjunctiva lines the outer aspect of the globe, while the palpebral portion covers the inside of the eyelids. Underneath the conjunctiva lie the episclera, the sclera and the uveal tissue layers [1]

Conjunctivitis occurs as epidemic disease especially in school age and child [2] care homes. Moreover bacterial conjunctivitis is one of the most frequent hospital-acquired infections [3, 4, 5, and 6]. When left untreated, Hospital Acquired Conjunctivitis can lead to severe sequelae, depending on the pathogen involved [7].

Conjunctivitis is an inflammation (redness) of the lining of the white part of the eye and the underside of the eyelid (conjunctiva). It can be caused by infection, allergic reaction, or physical agents like infrared or ultraviolet light [8]

Conjunctivitis is a common eye problem because the conjunctivae are continually exposed to microorganisms and environmental agents that can cause infections. Conjunctivitis can be acute or chronic. It can affect one or both eyes. It can be easily transmitted to others. Other name for conjunctivitis includes pinkeye and redeye [8].

Symptoms range from itching and redness to a mucous discharge [8]•

Antibiotics administered without susceptibility which lead to increase antibiotic resistant [8]. The aqueous layer contains the antimicrobial factors such as Lactoferrin, Lysozyme, IgA, Miscellaneous proteins [9].

A predictable set of organisms accounts for most cases of bacterial conjunctivitis in out-patients, so many physicians start therapy empirically without culturing the conjunctiva. But in the hospital the organisms and their antibiotic resistance patterns are more varied, so culturing the conjunctiva before starting

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broad-spectrum therapy may be warranted. For an outpatient with possible hyper acute conjunctivitis, it is reasonable to perform a Gram stain in the office if the facilities exist [10].

Unfortunately, antibiotic resistance is increasing even among out-patients. Susceptibility of the most common ocular pathogens to ophthalmic antimicrobial agents has dropped dramatically: *Streptococcus pneumoniae* and *Staphylococcus aureus* have developed high rates of resistance [10].

The most common infection of the eye is conjunctivitis most of these infections are caused by bacteria or by viruses [11].

Bacterial conjunctivitis usually quickly affects both eyes, whereas viral conjunctivitis is more likely to affect one eye only, and you don't usually get such a copious discharge [12].

Purulent conjunctivitis is usually indicative of bacterial conjunctivitis. The most common cases are *Staphylococcus aureus*, *Streptococcus pyogen*, *Streptococcus pneumonia*e, and Hemophilus spp; however the most destructive pathogens are *Gonococcus* and *pseudomonas aeruginosa*. *Gonococcus* in particular is often said to have a hyper acute presentation with severe inflammation and discharge developing over 12h [13].

Acute and chronic conjunctivitis associated with staphylococci, whereas recurrent infections associated with peliphiritis reaction in the eye [1].

Symptoms of bacterial conjunctivitis include a plus-like discharge and crusty eyelids after awakening [8].

Non purulent conjunctivitis (viral conjunctivitis) in contrast to bacterial, viral conjunctivitis tends to have a more watery discharge [13].

People can develop conjunctivitis by coming into direct contact with the tears or secretions from the eyes of an infected person and then touching their own eyes. Some viral particles may remain viable on surfaces for up to 49 days. Also conjunctivitis, when associated with an upper respiratory infection (common cold), can be spread by droplets (e.g., coughing, sneezing) [14] Conjunctivitis affects people at any age but tends to occur in preschoolers and school-age children because of crowding and lack of good hand-washing and hygiene [14]. Based on inflammatory cells present, the clinician can determine the presumptive cause of an inflammation (bacterial, viral, or allergic) [15].

Antibiotic eye drops or ointments are given if bacterial infection is suspected [11]. Acute bacterial conjunctivitis is a common disorder in children below 6 years' of age. *H.influenzae* is the most common etiology of the "conjunctivitis-otitis syndrome."[11].

Coagulase negative Staphylococci which isolated from patient with purulent conjunctivitis were identified as *Staphylococcus epedermitidis*, *Staphylocoous warneri, Staphylococcus capitis, Staphylococcus hominis, Staphylococcus xylosus, Staphylococcus simulans, Staphylococcus equorum, Staphylococcus lugdunensis*, from all isolated 28 show resistant to Tetracycline and four Ciprofloxacin [16].

Fusidic acid is active in vitro against *Staphylococcus aureus* and most coagulase-negative staphylococci [17].

Tetracycline has a broad spectrum of activity that includes gram negative bacteria and gram positive bacteria [17].

In vitro susceptibility of the most common ocular bacterial isolates to several antibiotics was assessed to verify changing trends in antibiotic susceptibility over 15 year period. All culture positive for *staphylococcus aureus*, and coagulase negative *staphylococci*. Susceptibility test were performed against Tobramycin, Ciprofloxacin, and Chloramphenicol showed 95%, 95%, 90% respectively [18].

Tobramycin inhibit protein synthesis by binding to protein receptor on small ribosomal subunit. This process interrupt initial formation of protein synthesis complex, accurate reading of mRNA code and so it inhibit protein formation [17].

Ciprofloxacin bind to and interfere with DNAase enzyme which is involved in regulation of DNA super twisting which is process that essential for DNA replication and transcription [17].

Chloramphenicol inhibit protein synthesis by binding to large ribosomal subunit this binding inhibit the addition of new amino acid to the growing polypeptide chain. It is highly active against gram negative and gram positive bacteria [19].

Methods:
A cross sectional study conducted in Abdalfadeel ALmath National Ophthalmic center – Khartoum (Sudan).

Study population:
A fifty eye swabs were collected randomly from conjunctivitis patients of different sex and age groups.

Sample collection:
Purulent material of the lower surface of the eye lid was collected on sterile cotton swab.

About 50 samples were collected from patients who diagnosed by physician as having bacterial conjunctivitis. Two swabs were collected from each case. One of the two swabs was dipped into brain heart infusion broth, and the other was taken for Gram and Geimsa smear.

Identification of isolated bacteria and sensitivity to antibiotics:
All bacteriological tests and sensitivity tests were done according to Cheesbrough Monica1991 [20], Barrow and Feltham 1993 and [21] Awad sheriff 2008 [22].

Culturing of samples:
All collected samples were cultured directly on brain heart infusion broth by placing the swab inside the plain container, the container was tightly covered, after incubation 24hours at 37ºC, sub-cultured on blood and chocolate agar to primary isolation. The inoculated plates were then incubated for 24 hours at 37ºC.

Purification:
All isolated bacteria were purified by several sub-culturing from single well-isolated colony. The purity of the culture was checked by examine gram stained smear. The pure colony was then used for studying cultural and biochemical characteristics of the isolates.

Identification of bacteria:
This including stains reaction, organism morphology, growth condition, colonial appearance on different media, and biochemical characteristics.

Geimsa staining technique: It was done to assist in identification of inflammation cells.

Smear preparation:-
-The swab was smeared directly into clean dry microscopically slide, and allowed to dry.

-The dried smear was fixed with absolute alcohol for three min-

utes, and allowed to dry.

Staining procedure:
The fixed smear was covered with10% diluted Geimsa solution for ten minutes, then examined with oil emersion lens.

Antimicrobial susceptibility tests:
It was carried out by disc diffusion method, normal saline suspension of tested organism was prepared by comparing the turbidity with Mcferland standard, bacterial suspension was inoculated on Mueller Hinton agar by swabbing all agar surface with cotton swab, single several antibiotic discs were then placed on agar surface with sterile forceps, after 24hours incubation at37°C the inhibition zone was measured by ruler in millimeter, then compared with incorporated chart [20, 23].

Results:
Isolated bacteria:
Out of 50specimens, 45 were showed positive growth, and 5 were showed no growth (table 1)

Table (1): Percentage of positive and negative growth:

Specimen	Number	Percentage
Positive growth	45	90
Negative growth	5	10
Total	50	100

Different types of isolated bacteria:
There are various microorganisms were isolated from collected specimens as showed in table (2):

Table (2): Percentage of isolated organisms:

Microorganisms	Number	Percentage
Staphylococcus aureus	5	11
Staphylococcus gallinarum	3	6.7
Staphylococcus simulans	2	4.5
Staphylococcus sciuri	2	4.5
Staphylococcus saccharolyticus	3	6.7
Staphylococcus cohnii	1	2.2
Staphylococcus schleiferi	5	11
Staphylococcus intermedius	2	4.5
Staphylococcus saprophyticus	1	2.2
Staphylococcus xylosus	4	8.9
Staphylococcus carnosus	1	2.2
Staphylococcus kloosii	2	4.5
Staphylococcus hyicus	1	2.2
Staphylococcus caprae	2	4.5
Staphylococcus hominis	6	13.4
Neisseria gonorrhea	5	11

Distribution of conjunctivitis according to the age of the patients:
The percentage of infection was higher in child more than adult neonate and infant (Table 3).

Table (3): Percentage of patients' age groups:

Age group	Number	Percentage
Neonate &infants	7	14
Children	23	46
Adults	20	40
Total	50	100

Distribution of conjunctivitis according to the predisposing factors:

The most cases were non immune compromise as showed in table (4)

Table (4): Percentage of predisposing factors:

Predisposing factor	Number	Percentage
Immune compromised patient	2	4
Non immune compromised patient	48	96
Total	50	100

Onset of disease:
Conjunctivitis occurs as acute form in most cases. (Table 5)

Table (5): Percentage of the onset of disease.

Onset of disease	Number	Percentage
Acute	40	80
Chronic	5	10
Recurrent	5	10
Total	50	100

Relation between onset and type of organism:
There is a differ of microorganism depend on onset of a disease as exhibited on table (6)

Table (6): Organisms related to the onset of the disease.

Onset of disease	Organisms
Acute	***Staphylococcus aureus*** ***Staphylococcus gallinarum*** ***Staphylococcus simulans*** ***Staphylococcus sciuri*** ***Staphylococcus saccharolyticus*** ***Staphylococcus schleiferi*** ***Staphylococcus intermedius*** ***Staphylococcus haemolyticus*** ***Staphylococcus xylosus*** ***Staphylococcus kloosii*** ***Staphylococcus hyicus*** ***Staphylococcus caprae***
Chronic	***Staphylococcus hominis*** ***Staphylococcus schleiferi*** ***Staphylococcus carnosus*** ***Neisseria gonorrhoeae***
Recurrent	***Staphylococcus cohnii*** ***Staphylococcus gallinarium*** ***Staphylococcus xylosus*** ***Staphylococcus saprophyticus*** ***Neisseria gonorrhoeae***

Antibiotic administration:
There were near to half percentage of patient administered antibiotic (Table 7)

Table (7): Percentage of patient administered antibiotic.

The patient condition	Number	Percentage
Patient a demonstrated antibiotic	18	36
Patient non a demonstrated	32	64
Total	50	100

Antimicrobial sensitivity:
All Isolates show sensitivity to Chloramphenicol, Ciprofloxacin and Tobramycin, and mild resistant to Tetracycline, and Fusidic acid (Table 8)

Table (8): Percentage of susceptibility of isolates to different antibiotics.

Type of antibiotic	Sensitivity %	Resistant %
Chloramphenicol	100	0
Ciprofloxacin	100	0
Tobramycin	100	0
Tetracycline	95.5	4.5
Fusidic acid	86.0	14.0

Discussion:
In this study we found that most specimens with predominant neutrophil show positive growth. This agree with Haesaert Susan, 1993 [15] who stated that Geimsa stain was used to identify the inflammatory cells present to determine the pre-

sumptive cause of an inflammation (bacterial, viral, or allergic).

In our present study some cases which diagnosed by physician as a viral conjunctivitis showing predominant neutrophil with lymphocyte and give positive growth we suggest that bacterial conjunctivitis appear as a secondary infection.

Some specimens which collected from patient administered antibiotic showing predominant neutrophil with negative growth; we suggest that the antibiotic affect the bacterial growth.

The most common causative agent was *staphylococcus* species this agree with Turkington and Ashby . 2007 [11] who stated that most of the conjunctivitis caused by *Staphylococcus* species.

Neisseria gonorrhoeae isolate from only neonate and infant of mother having gonorrhea, this agree with Turkington carol and Ashby B.L 2007 [11] who stated that neonate contact type of conjunctivitis is from their mother called gonococcal conjunctivitis.

conjunctivitis was more common in children rather than adult, this agree with Elmer et al 2006) [14] who stated that Conjunctivitis affects people at any age but tends to occur in Preschoolers and school-age children because of crowding and lack of good hand-washing and hygiene, and agree with Rose et al 2005[24], Kowalski and Dhaliwal, 2005[25] and Høvding, 2008[2].

The same causative agent was isolate from different members in a family; Tran Mai 2005 [8] also stated that conjunctivitis transmitted by contact.

Conjunctivitis occurs as acute form in 80% of cases, 10% as chronic form with *Staphylococci* being the most causative agents as Garry and Richard 1998 [1] who stated that acute and chronic conjunctivitis associated with *Staphylococci*.

All isolates show sensitivity to Chloramphenicol, Tobramycin and Ciprofloxacin, this agree with Gilbert Smolin *et al* 2005 [18] who stated that all culture positive for *staphylococcus aureus,* coagulase negative staphylococci are sensitive when performed against Tobramycin, Ciprofloxacin and Chloramphenicol showed 95% , 95% , 90% respectively.

Most isolates were sensitive to tetracycline, whereas patients who administered tetracycline still having the disease, this may be due to that antibiotic activity differs in vivo and vitro, or due to that patients use treatment without prescription.

Conclusion:

The recent study indicated that the bacterial conjunctivitis was commonly caused by *Staphylococcus species*; also *Neisseria gonorrhea* was isolated from infant of mother having gonorrhea.

Sensitivity tests indicated that Chloramphenicol, Ciprofloxacin, and Tetracycline were more effective than other drugs used against isolates identified in this study.

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