AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Microbiology and Antibacterial Susceptibility Pattern of Suppurative Keratitis Pathogens in Baghdad City

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ABSTRACT

Eye infections caused by pathogenic bacterial agents have an interesting social history, often with somewhat unsavoury associations. Corneal scrapings were obtained from 100 hospitalised patients from eye hospitals of Baghdad city, Iraq and inoculated directly onto enriched and differential culture media. Subcultures were performed on selective media. The necessary biochemical tests were conducted and the organisms identified using standard procedures. Susceptibility of isolated pathogens to commonly-used ocular antibacterial was examined using Kirby-Bauer's standard disc diffusion technique. The results were showed that 38 different organisms from total 100 corneal scraping samples of suppurative keratitis were isolated. Gram-positive bacteria accounted for 23(60.5%) and gram-negative bacteria for 15(39.4%). *Staphylococcus aureus* 11(28.9%), *Strep. Pneumoniae* 8(20.05%), *Pseudomonas aeruginosa* 6(15.7%), *Staph. epidermidis* 4(10.5%), *Klebsiella pneumoniae* 4(10.5%), *E. coli* 3(7.8%) and *Moraxella catarrhalis* 2(5.2%) were the most commonly-isolated organisms. In susceptibility testing, Gentamycin had significant effects against Gram positive bacteria which constituted the majority (82.5%) of isolates. Besides the coverage of this agent against Gram positive and negative organisms was also significant antibacterial activity that ranged from 50-100% sensitivity. Ofloxacin showed highly inhibition rate (75-100%) against all isolated pathogens except *Moraxella catarrhalis* (100%) resistance. Ampicillin coverage of against *Moraxella catarrhalis* 2(100%) and *E. coli* 2(66.6%). All the tested gram-positive cocci Staph. Species showed highly resistance to Chloramphenicol range from 90.9-100%. The coverage of Vancomycin against *Staphy. aureus* was 7(63.6%), *Moraxella catarrhalis* 2(100%), but all the isolates of *E. coli* were resistant to Vancomycin. Susceptibility analysis revealed that antibiotic with the greatest coverage was Gentamycin (82.5 % of 38 isolates). Gentamycin also had good coverage against Gram-positive cocci, which constituted the majority (100 %) of suppurative keratitis isolates.

Keywords: antibiotic susceptibility, bacteria, ocular infection, susceptibility patterns.

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Received 26 March 2012, Accepted 13 April 2012

Please cite this article in press as: Jebur MS *et al.*, Microbiology and Antibacterial Susceptibility Pattern of Suppurative Keratitis Pathogens in Baghdad City. American Journal of PharmTech Research 2012.

INTRODUCTION

Eye is a complex and sensitive organ, which is also more vulnerable to trauma and infections.[1] Infection of eye leads to conjunctivitis, Keratitis, endophthalmitis and other infections, which responsible for increased incidence of morbidity and blindness worldwide.[2,3]

Bacterial Keratitis refers to bacterial infection of the cornea. The surface epithelium, the mechanical properties of the lids and bioactive components of the tear film provide an effective barrier to infection. Infection may occur when these barriers are compromised and may then lead rapidly to ulceration of the cornea with resultant surface irregularity or corneal scarring, with associated loss in visual infection.[4] Suppurative Keratitis can cause corneal opacity and perforation, which leads to severe visual loss and is the second common cause for blindness in developing countries. [5,6] Bacterial Keratitis, Whose hallmark is the corneal ulcer, is usually associated with extended-wear soft contact lenses. [7] This condition progresses rapidly and cause substantial visual impairment, even in minor cases. [8] Also bacterial Keratitis is usually due to *Pseudomonas (Ps.) aeruginosa*, *Streptococcus(Strep.) pneumoniae* and rarely *Staphylococcus(Staph.) aureus*. [9] In North America, the most causative pathogens in corneal infections are *Staph. aureus* and *Staph. epidermidis*. [10,8] *Ps. aeruginosa* and *Staph.aureus* frequently cause severe Keratitis that may lead to progressive destruction of the corneal epithelium and stroma. [11,12]

Moraxella is a true ophthalmic pathogen, an "eye bug" par excellence that can occasionally be found colonizing the nasopharynx and on other mucous membranes. [13]

Successful treatment of ocular infection, including bacterial keratitis, requires multiple administrations of antibacterial agents to maintain drug concentration in the corneal tissue high enough and for a sufficient period of time to have useful antibacterial effect. [14]Because of anatomical features of corneal tissue, pathogenesis of bacterial invasion with multidrug resistance and fastidious microorganisms, it is useful to detect the susceptibility of these pathogens to antibacterial commonly-used in suppurative kerati The purpose of this study was to document the spectrum of organisms causing Suppurative bacterial Keratitis of the eye through isolation and diagnosis of causative bacterial pathogens in Baghdad eye infections, also to determine and advocate the appropriate antibacterial for their treatment.

MATERIALS AND METHODS:

1- Patients(specimens collection):

One hundred corneal scrapping samples were collected from patients suffering from Suppurative Keratitis, during March to August 2010 from eye hospitals of Baghdad city. All patients examined and diagnosed by ophthalmologist as a suppurative keratitis. Corneal scrapping has been taken, using slit lamp biomicroscope in an aseptic manner. Scrapping was done with the help of 4% Lignocaine and sterile bard parker blade(No.15). [15]

2- Cultures:

Corneal scrapping samples were inoculated on Blood agar, Nutrient agar, MacConkey agar, Manitol Salt agar and Chocolate agar (Hi-Media, India).The inoculated plates were incubated aerobically and anaerobically (at 5% - 10% CO_2 atmosphere) for 24 hrs. and 37°C. [16]

3- Identification of isolates:

The isolates were identified by various bacteriological tests, though colony characteristics, microscopically cells morphology, gram stain and necessary biochemical tests. [17,18,19] Biochemical tests were included catalase test, oxidase test, coagulase test, carbohydrate fermentation test, indol test, methyl red test, voges proskuer test, citrate utilization test, urease test, optochin sensitivity test, bile solubility test, nitrate reduction test, DNAase test and butyrate esterase production test. [20,19,17]

4- Antibacterial susceptibility test:

The isolated pathogens were further tested for their antibacterial susceptibility pattern by Kirby-Bauer's standard disc diffusion technique. [20] Control strains of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used for the standardization of Kirby-Bauer disc diffusion test. [22]

Antibacterial disc(Hi-Media, India) as National Committee for Clinical Laboratory Standards (NCCS) [23] were included Ampicillin (AMP) 10mcg, Chloramphenicol (C) 25mcg, Tetracycline(TE) 10mcg, Ciprofloxacin(CIP) 10mcg, Gentamicin(GEN) 30mcg, Erythromycin(E) 10mcg, Ofloxacin(OF) 2mcg, Ceftriaxone(CTR) 10mcg, Co-Trimoxazole(COT) 25mcg (Trimethoprim / Sulfamethoxazole 23.75 / 1.25), and Vancomycin(VA) 10mcg. [20,22] Mueller Hinton agar medium was used for susceptibility test. The results were interpreted as weather the isolated pathogen was sensitive or resistant to antibacterial agents[20], according the diameter zone of inhibition in mm listed in Biosciences Catalogue of Hi-Media Company 2010-11.

5- Statistical analysis:

To determine the statistical significance differences of variables, SPSS program were used. F test and Chi-square test were used to explain the significant differences between bacterial isolates

and antimicrobial susceptibility of agents. Probability value less than 0.05 was considered as statistically significant (*), while P-value more than 0.05 was considered as statistically not significant. Also the was used mean value and percent for appear the incidence of bacterial distributions. [24]

RESULTS AND DISCUSSION:

Suppurative Keratitis infection was develops from acute bacterial keratitis infection. However, the different pathogens able to invade the corneal of eyes and initiate episode of acute bacterial keratitis, which become after several complications to suppurative cases. For this progression of the disease all of us aware that suppurative keratitis is a great threat of eyes, that in turn pose a great problem in its treatment.

A total of one hundred corneal scrapping samples were collected from investigated and diagnosed patients as a suppurative keratitis cases, during the course of the present study. There were only 26(26%) cases showed positive bacterial culture with statistically significant differences P<0.05 compared to negative bacterial culture 74(74%). **(Figure.1)** Among positive cases, there were 20(76%) cultures had single bacterial growth with also statistically high significant differences P<0.05 to mixed bacterial growth which was 6(23.07%). **(Table.1)**

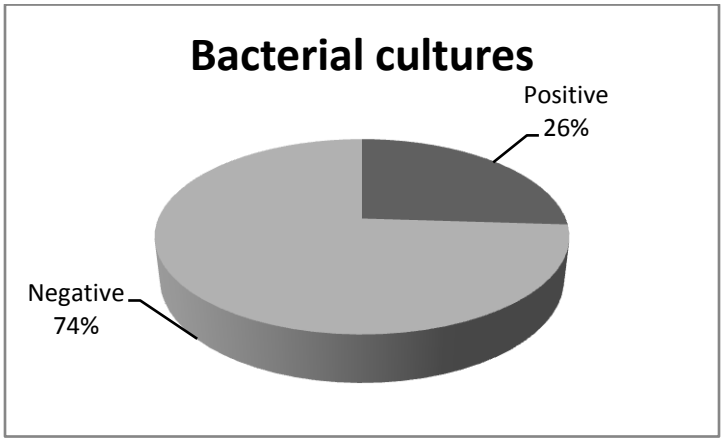

Figure.1: Distribution of bacterial cultures.

Table.1: Distribution of cases and cultures.

Culture	Samples	% culture	Mixed cultures	Single cultures	Total isolates	G+ Bacteria	G- bacteria
Positive	100	26(26%)	6(23.07%)	20(76%)	38(146%)	23(60.5%)	15(39.4%)
Negative	100	74(74%0	-	-	-	-	-

The positivity rate of bacterial culture keratitis in our study was 26% (Table.1) agreed to different studies by various authors, for that Khosravi *et al.* [25] 22%, Leck *et al.* [26] 23.9%, Das *et al.* [27] 29% and Sherwal *et al.* [15] 20.84%. This moderate percent of occurrence is essential to

explain that suppurative keratitis cases are important due to their prognoses which lead to severe visual loss and blindness to infected patients. [5,6]

The results were showed that out of 26 positive cases there were 38(146%) isolates of different bacterial pathogens identified. In total, 38 various organisms were isolated from suppurative keratitis patients, Gram positive bacteria accounted for 23(60.5%) of all bacterial isolates and Gram negative bacteria for 15(39.4%) of all bacterial isolates with highly significant differences P<0.05(Table.1)

Results of present study noticed that there were 38(146%) isolates caused bacterial suppurative keratitis(Table.1), whereas Gram positive bacteria accounted 23(60.5%) in comparative to Gram negative, which were 15(39.4%)from total bacterial causes. Many studies in this direction are available in past, [26,27,28] that reported Gram positive cocci are still the most common isolates among known and opportunistic pathogens of suppurative keratitis infection. Several other studies in India and other parts of the world have shown similar results inferring Gram positive cocci as primary causes of bacterial keratitis. [5,29,30] Also results of identification and diagnosis of bacterial cultures showed that the most common suppurative keratitis pathogen was *Staph. aureus* 11(28.9%), with high significant differences P<0.05 to all other isolates, followed by *Strep. Pneumoniae* 8(21.05%) also with significant differences P<0.05 to rest isolates.(Table.2) The third most common isolate was Gram-negative bacteria of *Ps. aeruginosa* 6(15.7%).(Table.2) *Staph. epidermidis* and *Klebsiella pneumoniae* were showed less prevalent bacterial identification 4(10.5%) for each one. Thereas E. coli and Moraxella catarrhalis were 3(7.8%) and 2(5.2%) respectively, with low significant differences P<0.05 to the prevalence of other isolated pathogens. **(Table.2)**

Table.2: Distribution of bacterial strains isolated from Suppurative Keratitis.

Organism(s)	Total No. of isolated recovered	% of isolates
Staph. aureus *	11	28.9
Strep. Pneumoniae *	8	21.05
Ps. aeruginosa *	6	15.7
Staph. epidermidis	4	10.5
Klebsiella pneumoniae	4	10.5
E. coli *	3	7.8
Moraxella catarrhalis *	2	5.2
Total	38	99.65

* Statistically significant differences P<0.05.

In present study, *Staph. aureus* was the most commonly isolates of suppurative keratitis pathogen 11(28.9%), followed by *Strep. Pneumoniae* 8(21.05%), *Ps. aeruginosa* 6(15.7%),

4(10.5%) for each *Staph. epidermidis* and *Klebsiella pneumoniae*, E. coli 3(7.8%) and *Moraxella catarrhalis* 2(5.2%). It accounted 146%(38 of 26 positive bacterial culture) from all suppurative keratitis pathogens isolated during the study period. This finding of bacterial isolation and diagnosis is similar to Shyam *et al.* [22)] study, who found among the 199(29.6%) culture positive cases, thirteen different bacterial pathogens were identified. *Strep. pneumoniae* was the most prevalent (40%) followed by *Staph. aureus* (20%), *Ps. aeruginosa* (4%), E. coli (2%), *Klebsiella pneumoniae* (1.5%), *Moraxella spp*. (1%) and others. [8-12] We believe that these pathogens are the most common in bacterial keratitis cases and these variations of isolation percent is due to laboratory sampling, bacterial cultures, isolation technique, geographical distribution, patient population managements, environmental contamination and many other risk factors. [31,32,33]

Study of Sherwal *et al.* [15] reported that the Gram positive cocci composed (52%) of bacterial keratitis and (42%) by Gram negative bacilli. Among Gram positive organisms *Staph. aureus* and *Strep. Pneumoniae* constituted (40%) and Gram negative bacteria *Ps. aeruginosa* (16%) of total positive bacterial cultures, in which these results are well correlated with our study. Leck *et al*. reported Strep. spp. (20%) and Staph. spp. (10%) in Ghana and from India he reported Strep. Spp. (46.8%), Staph. spp. (26.8%) and Ps. spp. (14.9%).[26] Moraxella catarrhalis is an important cause of lower respiratory tract infection, particularly in adults with chronic obstructive pulmonary disease. [34] In our study its isolated 2 from 38 isolates (5.2%), we thought that this low percent of isolation was due to its nosocomial properties[13], in other hand some competent consider it one of causative agents of ocular infection.[35]

Table.3: Susceptibility results of various bacteria isolated from Suppurative Keratitis commonly-used antibacterial.

Organism(s)	No. of isolates	No. sensitive(%) to antibacterial									
		AMP	C	TE	CIP	GEN*	E	OF	CTR	COT	VA
S. aureus	11	2(18.1)	1(9.09)	10(90.9)	6(54.5)	8(72.7)	7(63.6)	9(81.8)	2(18.1)	6(54.5)	7(63.6)
S pneumoniae	8	NS	5(62.5)	4(50)	5(82.5)	6(75)	1(12.5)	7(87.5)	6(75)	2(25)	3(37.5)
P.aeruginosa	6	NS	6(100)	NS	5(83.3)	3(50)	2(33.3)	5(83.3)	NS	NS	4(66.6)
S.epidermidis	4	1(25)	NS	3(75)	2(50)	3(75)	3(75)	4(100)	1(25)	3(75)	3(75)
K.pneumoniae	4	NS	3(75)	2(50)	3(75)	3(75)	4(100)	3(75)	2(50)	NS	2(50)
E. coli	3	2(66.6)	3(100)	NS	2(66.6)	3(100)	3(100)	3(100)	2(66.6)	3(100)	1(33.3)
M. catarrhalis	2	2(100)	2(100)	NS	2(100)	2(100)	2(100)	NS	2(100)	1(50)	2(100)

NS: No sensitivity, *Statistically significant differences P<0.05.

Isolation of *Klebsiella pneumoniae* was 4(10.5%) percent which disagree with results of Gichangi *et al.*, [36] who isolated this pathogen in (6.25%) percent from eye infection patients, whereas the study isolated *Staph. aureus* in (31.25%). The isolated pathogens were tested for their susceptibility towards commonly-used antibacterial agents for ocular infections explained

that *Staph. aureus* isolates which were the main prevalence organism in suppurative keratitis, appear highly significant (P<0.05)sensitivity to Tetracycline 10(90.9%) and 9(81.8%) for Ofloxacin, followed by 8(72.7%) to Gentamicin, 7(63.6%) to Erythromycin and to Vancomycin, 6(54.5%) to Ciprofloxacin and Co-Trimoxazole. Whereas it had appeared resistant to Ampicillin, Ceftriaxone 9(81.8%) and 10(90.9%) to Chloramphenicol. **(Table.3)**

Based on results from susceptibility testing (Table.3), *Strep. pneumoniae* isolates were recorded highly significant sensitivity(P<5.05) to Ofloxacin 7(87.5%), followed by Gentamicin and Ceftriaxone 6(75%), 5(62.5%) to Chloramphenicol and Ciprofloxacin. Streptococcal isolates were appeared highly resistant to Ampicillin 8(100%), Erythromycin 7(87.5%) and Co-Trimoxazole 6(75%). The main causative Gram negative bacteria suppurative keratitis *Pseudomonas aeruginosa* isolates were not sensitive to Ampicillin, Tetracycline, Ceftriaxone and Co-Trimoxazole. Whereas *Pseudomonas* isolates were resistant to Gentamicin and Erythromycin 3(50%) and 4(66.6%) respectively, but all isolates were showed significant sensitivity(P<0.05) to Chloramphenicol 6(100%) and 5(83.3%) to Ciprofloxacin and Ofloxacin, and 4(66.6%) to Vancomycin.(Table.3) Table.3 shows that in each drug group, the frequency sensitivity of Tetracycline, Gentamicin, Erythromycin, Co-Trimoxazole and Vancomycin(75-100%) were statistically significant higher sensitive than other antibacterial. P<0.05, while 4(100%) of *Staph. epidermidis* was resist to Chloramphenicol, 1(25%) to Ampicillin and Ceftriaxone, whereas 2(50%) the pattern of resistance was appeared to Ciprofloxacin. Highly significant sensitivity(P<0.05) of Erythromycin(4(100%), 3(75%) for each Ciprofloxacin, Gentamicin and Ofloxacin in *Klebsiella pneumoniae* isolates were recorded, but there were moderate sensitivity 2(50%) to Tetracycline, Ceftriaxone and Vancomycin, while these isolates were appeared highly significant resistant(100%) to Ampicillin and Co-Trimoxazole.(Table.3) Isolates of pathogenic *E. coli* showed higher significant sensitivity 3(100%) for Chloramphenicol, Gentamicin, Erythromycin and Co-Trimoxazole, but less sensitivity 2(66.6%) for Ampicillin, Ciprofloxacin and Ceftriaxone, also the study reported that there was no sensitivity to Tetracycline and 2(66.6%) to Vancomycin.(**Table.3)** Table.3 show that the frequency of Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Erythromycin, Ceftriaxone and Vancomycin were statistically significantly higher than other types of antibacterial. P<0.05, with 100% percent of sensitivity, whereas 50% to Co-Trimoxazole but other isolates were showed higher significant resistance 100% to Tetracycline and Ofloxacin. The results of present study was noticed that the mixed bacterial culture had high significant sensitivity to Ciprofloxacin, Gentamicin and Chloramphenicol.

Antibacterial are generally effective for treatment of suppurative keratitis cases. However, frequent indiscriminate use and widespread prescription of antibacterial has led to the development of resistance to many commonly-used antibacterial. [37,38,39] Based on the results from susceptibility analysis of suppurative keratitis pathogens isolated, the antibacterial with the greatest coverage was Gentamicin(50-100%) of 38 isolates of all bacterial types (Table.3), so Gentamicin had significant effects against Gram positive bacteria which constituted the majority (82.5%) of isolates in the study. Besides the coverage of this agent against Gram negative organisms was also significant antibacterial activity that ranged from 50-100% sensitivity percentage. [25] Some of the antibacterial used in present study had appeared highly effects against the isolated pathogenic agents, Ofloxacin showed highly inhibition rate (75-100%) against all isolated pathogens except *Moraxella catarrhalis* isolates (100%) resistance, this results were agree to mention of Donnenfeld *et al.* [40], who refer to usage of Ofloxacin in bacterial keratitis because of higher aqueous levels of it that can be attained with comparative dosing regimens. Synthetic Penicillin, Ampicillin was found drug of choice for different bacterial agents especially Gram positive bacteria. But during the last years, the incidence of resistance has been increased in many country because of population randomly administration, whereas the results of susceptibility explain this fact, which showed resistance of *Staph. aureus*, *Strep. pneumoniae*, *Ps. aeruginosa*, *Staph. epidermidis* and *Klebsiella pneumoniae*, while *Moraxella catarrhalis* appeared highly sensitivity 2(100%) and *E. coli* 2(66.6%), so that these results of susceptibility disagree to study done by Tamang *et al.* [20] Wide sensitivity of isolated pathogens to Chloramphenicol (Table.3) explain its drug of choice in ocular infections specially bacterial keratitis,[41] but Staph. species appeared resistance range from 90.9-100% that we thought is due to incorrect uses of Chloramphenicol to several bacterial diseases. The different sensitivity percent were seen in suppurative keratitis isolates to Ciprofloxacin, Tetracycline, Erythromycin, Ceftriaxone and Vancomycin, which concern to bacterial properties, [42,20,36] laboratory procedures, drugs formulation. [43,44,45]

Because of low activity of Co-Trimoxazole against isolates of present study, we believe that further studies are needed to confirm its efficacy on suppurative keratitis pathogens.So, we do not recommend treatment of bacterial keratitis and other ocular infections on an empirical basis, especially in the centres where ophthalmologists have access to microbiology facilities. An attempt should be made to identify the ocular pathogen and performing susceptibility testing. It should be borne in mind that these are *in-vitro* results and do not always mirror the clinical response to antibiotics due to a variety of reasons including direct topical delivery, corneal penetration of an antibiotic and host factors. [46]

CONCLUSIONS

The results were obtained from this study provide information that allows a clinician to make an informed decision when choosing an initial regimen for treatment of suppurative keratitis pathogens. Furthermore, the result of the present study provides susceptibility information for ocular pathogens which are resistant to commonly-used ocular antibacterial agents.

ACKNOWLEDGEMENT:

We thank the clinical staff at eye hospitals of Baghdad city for examining the patients and collecting the specimens, the technical staff of the Institute of Medical Technology Laboratory and the patients for their consent.

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