Journal of Pharmaceutical Research International

33(45A): 383-388, 2021; Article no.JPRI.69002
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Evaluation of Antibiotic Sensitivity Test against Ophthalmic Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Author RS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ASSR and SB managed the analyses of the study. Author VA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i45A32755
Editor(s):
(1) Prof. Hamdy A, Sliem, Suez Canal University, Egypt and Qassim University and EL-Jouf University, Saudi Arabia.
(2) Dr. Rafik Karaman, Al-Quds University, Palestine.
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Complete Peer review History: https://www.sdiarticle4.com/review-history/69002

Original Research Article

Received 02 May 2021
Accepted 15 July 2021
Published 02 October 2021

ABSTRACT

Ophthalmic infections can cause damage to the structure of the eye which can lead to vision loss and blindness if left untreated. Ophthalmic infection or eye infections are caused by exposure to bacterial, fungal viral and protozoan are common with frequently reported in Asian countries. In the present study, the external ocular infected samples collected from Thanjavur Medical College Hospital, Thanjavur. Seven strains were isolated from the external ocular infected samples and identified a standard manual of Determinative Bacteriology by Bergy's manual 12th edition. The commercial antibiotics and eye drops tested against *Bacillus cereus, B. subtilis, Escherichia coli*, *Klebsiella* sp. *Pseudomonas* sp. *Streptococcus* sp. and *Staphylococcus aureus.* The majority of the isolates were sensitive to tobramycin followed by moxifloxacin, gatifloxacin and ofloxacin. The resistant antibiotics are ciprofloxin and sensitive antibiotic was ampicillin was recorded with respective bacteria.

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Keywords: Ocular pathogen; commercial eye drops; antibiotics.

1. INTRODUCTION

The eye is a unique organ that is impermeable to almost all external organisms. The eye may be infected from external sources or through intra-ocular invasion of microorganisms carried out by blood stream [1]. continuous tear flow aided by the blink, reflex and mechanically washes are cleaned from the ocular surface and to prevent the external microbes. In addition lactoferrin, lysozyme, secretary immmunogloblin's and defines, which are high levels in tears, can specifically reduce bacterial colonization of the ocular surface [2]. Opportunistic microbes are caused to ocular disease and frequently affected the area of eye are conjunctiva, lid and cornea [3,4].

Ocular infections are common in most the developing countries in the world. Bacteria are generally associated with many types of infections such as conjunctivitis, keratitis, endophthalmitis, Blepharitis, orbital cellulitis, dacryocystitis, canaliculitis are common ocular infection [5]. Conjunctivitis is the most common cause of redeye and corneal ulceration is a major cause of mono ocular blindness in developing countries [6]. The most common causes of conjunctivitis are infective conjunctivitis is most commonly caused by bacteria and viruses. Viral conjunctivitis causes a watery discharge the bacterial conjunctivitis contains puss. Infective keratitis is a major cause of vision loss and blindness second to cataract[2]. Ocular trauma is a common predisposing factors of infectious keratitis in developing countries, whereas pre-existing ocular disease and wearing contact lens are common risk factor in developed countries [7]. Blepharitis is an inflammation of the eyelid margin [8]. Many factors determine the clinical outcome in microbes causing eye infection and the epidemiological patterns vary from one country to another and in different geographical areas in the same country [9]. The gram-negative endophthalmitis cases, *Pseudomonas aeruginosa*is the most commonly isolated organism [10].

2. MATERIALS AND METHODS

2.1 Sample Collection

Ocular infected samples were one time collection from Government Medical College Hospital, Thanjavur. The pus samples were collected from infected eye by sterile swab and Transfer to bacterial transport media. The collected samples were brought to the laboratory within 24hrsfor future analysis.

2.2 Isolation and Identification of Bacterial Pathogen [11]

The isolation of bacteria was done by the streak plate method of nutrient agar plate techniques and incubated for 37°C for 24 – 48hrs. They observed mixed colonies and was purified by repeated subculture on media originally used for their isolation. Pure culture was stored in nutrient agar slants for further tests.

The strains were identified by microscopic and biochemical studies of Gram's staining, motility, indole, methylred, Voges proskeaur, citrate, catalase, lactose fermentation, glucose fermentation and oxidase test were performed. The results compared to Bergy's manual of Determination of Bacteriology, 12th edition.

2.3 Determination of Antibiotic Susceptibility [12,13]

The bacterial pathogens were grown on nutrient broth (NB) at 37°C for 24 hours in incubation. A sterile cotton wool swab dipped into the 24 hours old bacterial suspension was spread evenly on the surface of the Muller Hinton agar plates and placing the antibiotics by discs diffusion test. The following antibiotics were used Ampicillin (10µg), Streptomycin (30µg), Chloramphenicol (30µg), Ciprofloxacin (30µg) and Ofloxacin (30µl), Gatifloxacin (30µl), Moxifloxacin (30µl), Tobramycin (30µl) eye drops were placed on the surface of the MH agar plates and incubated at 37°C for 24 hours. The diameter of inhibition zones formed surrounding each well inclusive of the diameter of the discs was measured.

3. RESULTS AND DISCUSSION

In the present study, seven species of bacteria was isolated from ocular infected samples. The isolate colonies were identified based on microscopic and biochemical characteristics. Most are gram-positive, motile and negative results observed in IMViC test (Table 1). This is in agreement with the results obtained [14,15,16].

S. aureus predominate in eyelid and conjunctiva infected organism and coagulase negative staphylococci in intra-ocular tissue infections. Acute and chronic infection of the eyelid margin and glands of the eyelid are reported predominantly by *S. aureus* and *S. epidermidis* [17]. In our investigation, the identified bacterial species are *Bacillus cereus, B. subtilis, Escherichia coli*, *Klebsiella* sp. *Pseudomonas* sp. *Streptococcus* sp. and *Staphylococcus aureus*. Esenwah[17] reported that 15% of his isolates were *E. coli* bacteria. *E. coli* had been reported to be responsible for certain ocular inflammations such as endophthalmitis, keratitis, conjunctivitis, blepharitis, orbital cellulitis and dacryocystitis [18].

In the present investigation, standard eye drop (Ofloxacin, Gatifloxacin, Moxifloxacin and Tobramycin) and antibiotic sensitivity test (Ampicillin, Streptomycin, ciprofloxin and chloramphenicol) were performed against ocular pathogens of *Bacillus cereus, B. subtilis, Escherichia coli*, *Klebsiella* sp. *Pseudomonas* sp. *Streptococcus* sp. and *Staphylococcus aureus.* The maximum zone of inhibition measured in *Bacillus cereus* (25.6±1.8mm) in ofloxacin, Klebsiella sp. (25.0±5.8mm) in Gatifloxacin, *Escherichia coli* (25.7±0.2mm), *Klebisella* sp. (25.0±5.8mm) and *Streptococcus* sp. (25.0±2.5mm) in moxifloxacin and *Escherichia coli* (30.0±0.5mm) are shown in Table 2. Similarly [19]. *E. coli* and *Staph. aureus* were sensitive to seven antibiotics namely Amikacin, Chloroamphenicol, Ciprofloxacin, Methicillin, Moxifloxacin, Ofloxacin, and Tobramycin. *P. aeruginosa* was also moderately resistant to fluroquinolone antibiotics such as ciprofloxacin and aminoglycosides antibiotics such as gentamicin and amikacin with percent resistance of 24, 24 and 23%, respectively [20].

Table 1. Biochemical characterization of bacteria from ocular infection

Strain	GS	M	I	MR	VP	Ci	Ca	L	G	O	Identification of bacteria
RSVA1	+	-	-	-	+	-	+	-	+	+	*B.cereus*
RSVA 2	+	+	-	+	-	-	+	+	+	-	*B.subtilis*
RSVA3	-	+	+	-	+	-	-	-	-	+	*E. coli*
RSVA4	+	-	-	+	-	+	-	-	+	-	*Klebsiella*sp.
RSVA5	-	+	-	+	-	+	-	-	+	-	*Pseudomonas* sp.
RSVA6	+	+	+	+	-	-	+	-	-	+	*Streptococcus* sp.
RSVA7	+	+	-	-	+	-	-	-	+	+	*S.aureus*

GS-Gram stain, M-Motility, I-Indole, MR-Methyl red, VP-Voges proskeaur, Ci-Citrate, Ca-Catalase, L-Lactose, G-Glucose, O-Oxidase

Table 2. Effect of commercial Eye drops for ocular infection

Ocular Pathgoen	Ofloxacin	Gatifloxacin	Moxifloxacin	Tobramycin
	Zone of inhibition (mm±SD)			
Bacillus cereus	25.6±1.8	15.0±4.6	25.0±0.5	25.0±5.6
B. subtilis	11.0±3.6	9.0±3.0	10.0 ±0.5	15.0±0.5
Escherichia coli	20.0±4.3	20.0±3.0	25.7±0.2	30.0±0.5
Klebsiellasp	22.0±9.6	25.0±5.8	25.0±3.0	20.0±4.6
Pseudomonas sp.	11.3±7.1	15.0±6.3	15.3±0.5	20.0±6.0
Streptococcus sp.	20.0±8.0	24.0±8.0	25.0±0.2	25.0±4.6
Staphylococcus aureus	12.0±5.8	14.0±4.6	15.0±2.5	25.0±0.5

Table 3. Determination of effect of antibiotics against ocular pathogenic bacteria

Ocular pathogen	Ampicillin	Streptomycin	Ciprofloxin	Chlorampenicol
	Zone of inhibition (mm±SD)			
Bacillus cereus	**-**	29±1.6	27.3±3.11	**-**
B. subtilis	**-**	19.6±6.3	37.0±12.3	24.0±7.6
Escherichia coli	**-**	**20±0.5**	32.6±8.5	18.6±6.2
Klebsiella sp.	**-**	**-**	22.6±7.5	18.0±6.0
Pseudomonas sp.	**-**	-	28.0±9.3	-
Streptococcus sp.	**-**	9.0±±3.0	26.0±8.6	**-**
Staphylococcus aureus	**-**	25±4.5	35.±5.0	-

The best available antibiotic for bacterial conjunctivitis was Moxifloxacin. Therefore, this antibiotic seems to be the best choice for patients with bacterial conjunctivitis of all ages. Although resistance is developing with older fluorquinolones (Ciprofloxacin, Ofloxacin) it is less common with newer fluorquinolones (Moxifloxacin, Gatifloxacin, Bestifoxacin) because, the newer fluoroquinolones exhibit balanced dual binding of these enzymes and require Multi-step mutations, whereas resistance to the older fluoroquinolones which typically target one enzyme in preference to the other, may require only a single such mutation [21, 22].

The effect of antibiotics sensitivity test was performed against *Bacillus cereus, B. subtilis, Escherichia coli*, *Klebsiella* sp. *Pseudomonas* sp., *Streptococcus* sp. and *Staphylococcus aureus* of ocular pathogens. Four commercial antibiotics as Ampicillin, Streptomycin, Ciprofloxin and Chloramphenicol were chosen for this antibiotic sensitivity study. The ciprofloxin antibiotics are more sensitive activity against *Bacillus cereus* (37.3±3.1mm), *B. substilis* (37.0±12.3mm), *Escherichia coli* (32.6±8.5mm), *Klebsiella* sp. (22.6±7.5mm) *Pseudomonas* sp. (28.0±9.3mm), *Streptococcus* sp. (26.0±8.6mm) and *Staphylococcus aureus* (35.0±5.0mm) and resistance to ampicillin antibiotics than compared to other antibiotics.

Some researchers reported that as streptomycin antibiotics showed the highest zone of inhibition against *S. aureus* (32mm), but lowest against *B. cereus* (27mm), at a similar concentration [23]. Shijila Rani et al. [24] reported that the ciprofloxacin antibiotics have been well documented in the high zone formation in *B. cereus,* coagulase-negative *Staphylococcus*, *E. aerogenes, E. coli, Enterobacter* sp., *K. pneumoniae, P. aeruoginosa, Proteus* sp., *Pseudomonas* sp., *S. aureus* and *S. typhi*. Ong et al. [25] evaluated that strongly susceptible to five antibiotics which include Amikacin, Kanamycin, Gentamycin, Norfloxacin and Tetracycline. Additionally [26,27,28] have all previously reported bacterial resistance against ampicillin, gentamycin, erythromycin, tetracycline & ciprofloxacin at different times. The reason why some of these *E. coli* isolates showed a high level of resistance to the antimicrobial agents used is an indication that these antibiotics have been abused, hence the possibility that they have acquired resistance.

4. CONCLUSION

In our study the concluded that the High antibiotic resistance to commonly prescribed antibiotics activity against ocular pathogen were observed Therefore, to prevent the increasing rate of antimicrobial resistance identification of bacteria through culture methods and conducting drug susceptibility test should be practiced as a routine diagnostic we recommend the identification of bacteria and drug susceptibility test as routine diagnostic procedure in every ocular infection to prevent increasing antimicrobial resistance rate.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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