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ANTIMICROBIAL ACTIVITY OF CERTAIN ANTIBIOTICS ON THE MICROBIAL ISOLATES OF KERATITIS PATIENT

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ABSTRACT

Microbial keratitis is a major cause of monocular blindness in developing countries. Keratitis caused by bacteria, viruses, fungi and parasites. To investigate the identification of bacteria and fungi from keratitis patients using certain antibiotics. The samples were examined in the Eye Clinic of Annal Gandhi Memorial Government, Hospital, Tiruchirappalli, India. All patients were examined with a slit lamp biomicroscope. The bacterial and fungal colonies were isolated in eye swabs of keratitis patients and were identified by using biochemical tests. In this study, the antimicrobial activity of several antibiotics was evaluated using micro dilution and antimicrobial susceptibility tested against clinically isolated microorganisms from keratitis patients. The antimicrobial activity for *Staphylococcus aureus, Streptococcus viridians, Pseudomonas aeuoroginosa, Staphylococcus epidermis, Candida albicans* and *Aspergillus tubingensis* against various antibiotics such as Gentamycin, Ampicilin, Tetracycline, Ciprofloxacin, Nystatin, Flucytosine, Amphotericin and Miconazole were studied. Most of the bacteria were sensitive to Gentamycin, Ampicillin, and Tetracycline whereas the fungi like *Candida albicans* and *Aspergillus tubingensis* were sensitive to Nystatin and Flucytosine. In the present study, all bacteria and fungi were found to be sensitive to Gentamycin, Ampicilin, Nystatin and Flucytosine.

KEYWORDS: Microbial keratitis, Bacteria, Fungi, Antibiotics.

INTRODUCTION

Keratitis is a common infectious disease of the cornea [1] that is potentially causes blinding. [2] All infections of the eye are very serious because opaque fibrous tissue or scar tissue may form on the cornea during the healing process and cause partial or total loss of vision. Keratitis is mostly caused by bacteria, viruses, fungi and parasites. These microorganisms are resistant and hence they were so called multidrug resistant (MDR) strains. The multidrug resistant strain of many microorganisms has revealed exploration of alternative antimicrobial agents. Hence there is an urgent need to find an alternative antimicrobial agent to treat these virulent microbial infections. Since the discovery of antibiotics and the advancement of medical technology, the incidence of microbial keratitis has been drastically reduced in developed countries. The incidence of microbial keratitis is still quite high in developing countries, mainly due to lack of medical awareness or in certain parts, due to inaccessibility to medical treatment. The present investigation is an attempt to study an *in vitro* antimicrobial activity of keratitis causing microorganisms with certain antibiotics like Gentamycin, Ampicilin, Tetracycline, Ciprofloxacin, Nystatin, Flucytosine, Amphotericin and Miconazole. Treatment was commenced empirically with broad spectrum topical antibiotics immediately after the diagnosis was made.

MATERIALS AND METHODS

SAMPLE COLLECTION

In this study the microorganisms were isolated from keratitis patients visited the Eye clinic of General Hospital of Tiruchirappalli during 2011-2012. All patients were examined under a slit-lamp biomicroscope by an ophthalmologist. Corneal scrapings were collected after instillation of 4% lignocaine without preservative under aseptic conditions from each ulcer by an ophthalmologist using a sterile Bard Parker blade (No 15). Scrapings were performed under magnification of slit-lamp or operating microscope. Leading edge and base of each ulcer were scraped initially and the material obtained were directly inoculated onto the surface of solid media such as sheep blood agar, chocolate agar and Sabouraud Dextrose Agar (SDA) in a row of C- shaped streaks and also deep inoculation in the liquid media such as Brain Heart Infusion (BHI) broth without Gentamicin sulphate and Thioglycollate medium. Subsequent scrapings were spread onto labelled slides in a thin, even manner for 10 % potassium hydroxide (KOH) wet mount and Gram staining. Control patients were defined as patients who had epithelial defects of non-infectious or infective keratitis and were age matched.

IDENTIFICATION OF BACTERIA

Both morphological and biochemical tests were carried out for bacterial identifications.

MORPHOLOGICAL TEST

i) Gram Staining: This method was used to identify and differentiate the Gram-positive and Gram-negative bacteria. A loop full sample of the bacteria was mixed with a drop of distilled water on a microscope slide and allowed to air dry. The smear was covered with crystal violet and allowed to stand for 20 seconds. The stain was briefly washed off using a wash bottle of distilled water. Excess of water was drained off. The smear was covered with Gram's iodine solution for 30 seconds. Later it was washed off and the slide was hold 45- degree angle and allows the 95% alcohol to flow down the surface of the slide until the alcohol was colourless as it flows from the smear down the surface of the slide. Decolourization was stopped by washing the slide with gentle smear of water. Then the smear was covered with safranin stain for 1 minute. The slide was washed gently for few seconds then blotted to dry with bibulous paper and air-dried. The slides were examined under oil immersion. [3],[4],[5]

BIOCHEMICAL TESTS

The biochemical tests. [6] were performed in order to identify the bacteria present in the eye swabs of keratitis patients. Some of the tests provided immediate results while others had to be incubated for a period of time.

ISOLATION OF FUNGI FROM SAMPLE

Samples were inoculated on Poatato Dextrose Agar (PDA) medium resulted in fast-growing colonies with cottony aerial mycelia. The isolates were sub-cultured on malt extract agar plates for morphological identification. Fungi were identified by their colony characteristics on PDA by their macroscopic and microscopic appearance in Lactophenol cotton blue. For the purposes of molecular identification, mycelia were grown on liquid media containing 0.5% yeast extract, 0.5% peptone and 1% glucose for 1 day and were subjected to DNA isolation. Fungal cultures from cases with smears negative for fungi were discarded as possible contaminants, although Chin *et al*., (1975) pointed out that negative smears and cultures do not necessarily rule out fungal infection. [7]

ANTIMICROBIAL SENSITIVITY TEST

There is a need to integrate medicine and innovative technology in our public health system to provide rapid, efficient, accurate, and cost-effective results for identification and

antimicrobial susceptibility testing (AST) of pathogens. The isolates from the keratitis, were processed directly from the primary plates, and purity testing was done simultaneously by standard methods. Identification was done by conventional methods and was confirmed on the basis of results obtained by performing routine biochemical tests and AST was performed by the disc diffusion test based on Kirby-Bauer method.

Antibiotics susceptibility testing of each isolated pathogen was done using routine antibiotics discs Gentamycin, Ampicillin, Tetracycline, Co-trimaxazole, Ceftriaxone, Cephalotinn, Cefotaxime, Kananmycin according to disc diffusion technique by Kirby Bauer method on Muller Hinton agar (MHA).

PROCEDURE

Muller Hinton agar plates were prepared and the test microorganisms were inoculated by the spread plate method. Filter paper discs of approximately 6 mm in diameter were soaked with 15µl of the antibiotics solution and placed on the preciously prepared agar plates. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they were no closer than 24mm from each other centre to centre. The agar plates were incubated at 37°C. After 16 to 18 hrs. of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc where the chloramphenicol was used as control. [8]

This method was efficient for rapid identification and antimicrobial susceptibility testing of bacteria and fungi, which were critical, helps in adequate therapy and patient care. By providing faster results, it decreases antibiotic consumption and improves patient care.

RESULTS

IDENTIFICATION OF BACTERIA BY LABORATORY DIAGNOSIS

Laboratory diagnosis can be made by means of smear, staining, biochemical tests, fungal culture and microscopically identification. The results observed previously, bacterial colonies can differ greatly in their morphologies. These differences can help us in identifying different species of bacteria. Likewise, bacterial species differ in their cellular morphologies and staining properties. Again, these differences can be used to aid in identifying different species.

The bacterial culture plates containing *Streptococcus viridans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were isolated from keratitis patients and identified by using biochemical tests. The results revealed that they were cocci and rod shaped, Gram positive and Gram negative bacteria and also it had glucose, lactose, sucrose fermentations (acid & gas), catalase enzyme activity (degradation of hydrogen peroxide) and non-acidic in nature.

IDENTIFICATION OF FUNGI BY MICROSCOPICAL VIEW

Direct microscopic evaluation is the most valuable and rapid diagnosis for the detection of fungal filaments in corneal scrapings. Fungus grows within 48 to 72 hours in blood agar and Sabouraud dextrose agar kept at room temperature. The positive fungal culture with lactophenol cotton blue staining showed fungal elements in keratitis patients. *Aspergillus sp.* and *Candida sp*, were isolated. The results for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of KOH preparation, Gram stain, and culture for identification of fungi.

ANTIBIOTIC RESISTANCE IN CAUSATIVE MICROORGANISM

The antimicrobial agents available today are mostly microbistatic, requiring a prolonged course of therapy. The antimicrobial activity for *Staphylococcus aureus*, *Streptococcus viridians*, *Pseudomonas aeuoroginosa*, *Staphylococcus epidermis*, *Candida albicans* and *Aspergillus tubingensis* against various antibiotics such as Gentamycin; Ampicilin; Tetracycline; Ciprifloxacin; Nystatin; Flucytosine; Amphotericin; Miconazole were studied. From the results it was observed that the antibiotics showed their capacities to inhibit the growth of these bacteria that were quite resistant to antibiotics, as indicated in Table 1. The antifungal activity of the Nystatin and Flucytosine were found to be nearly similar since the Nystatin is the drug of choice for *Candida albicans*. The current work has shown that Gentamycin, Ciprifloxacin, Ampicilin, Nystatin and Flucytosine are the potential source of antimicrobial agents and their activity against various clinical isolates may be sufficient to perform further studies for isolation and identification for active principles.

Antimicrobial activities of microorganisms were evaluated based on the diameters of clear inhibition zone surrounding the paper disks. If there is no inhibition zone, it is assumed that there is no antimicrobial activity. In *Staphylococus aureus* and *Staphylococcus epidermis*, the diameter of inhibition zone of Gentamycin is larger than that of other antibiotics. In *Streptococcus viridians* indicating Ciprofloxacin is more susceptible than other antibiotics. In

Pseudomonas aeruginosa the diameter of inhibition zone of Ampicillin is larger than that of other antibiotics. In *Candida albicans* and *Aspergillus tubingensis*, the diameter of zone of inhibition with Nnystatin and Flucytosine is larger than other antibiotics.

In infectious corneal eyes, antibiotic susceptibility testing showed that bacterial isolates sensitive to the Gentamycin, Ampicillin, Tetracycline and Ciprifloxacin antibiotics. The majority of bacteria isolated in the study were sensitive to either Ampicillin and/or Gentamicin. Most of the bacteria were sensitive to gentamycin, ampicillin, and tetracycline antibiotics whereas the fungi were sensitive to Nystatin and Flucytosine antibiotics. These findings, most of the cases support the use of the Gentamycin and Nystatin for the prevention and treatment of serious ophthalmic infections (eg, keratitis, endophthalmitis) caused by susceptible bacteria.

Table 1 Antimicrobial test against isolated bacteria and fungi

Bacterium	**Zone of inhibition (mm)**					
	+ve control (1)	**-ve control (2)**	**GM (3)**	**AM (4)**	**TE (5)**	**CIP (6)**
Staphylococus aureus	10	-	11	11	10	11
Streptococcus viridians	13	-	10	11	10	10
Pseudomonas aeruginosa	16	-	14	15	10	11
Staphylococcus epidermis	12	-	11	10	10	11
Fungus	**Zone of inhibition (mm)**					
	+ve control (1)	-ve control (2)	N (3)	F (4)	A (5)	M (6)
Candida Albicans	12	-	12	11	10	11
Aspergillus tubingensis	12	-	11	12	10	11

GM – Gentamycin; AM – Ampicilin; TE – Tetracycline; CIP- Ciprifloxacin; N- Nystatin; F- Flucytosine; A - Amphotericin; M - Miconazole.

DISCUSSION

Microbial keratitis needs to be treated with antibiotics. Depending on the severity of the infection, an oral antibiotic may be prescribed along with an antibiotic ointment or eye drops. Infectious keratitis can progress rapidly, and generally requires urgent antibacterial and antifungal therapy to eliminate the pathogen.

Several hundreds of compounds with antibiotic activity have been isolated from microorganisms over the years, but only a few of them are clinically-useful. The reason for this is that only compounds with selective toxicity can be used clinically. The selective toxicity of antibiotics means that they must be highly effective against the microbe but have minimal or no toxicity to humans. Antibiotics may have a cidal (killing) effect or a static (inhibitory) effect on a range of microbes. The range of bacteria or other microorganisms that is affected by a certain antibiotic is expressed as its spectrum of action. Antibiotics effective against prokaryotes that kill or inhibit a wide range of Gram-positive and Gram-negative bacteria are said to be broad spectrum. If effective mainly against Gram-positive or Gram-negative bacteria, they are narrow spectrum. If effective against a single organism or disease, they are referred to as limited spectrum.

In our research, the preliminary investigation of antimicrobial activity for *Staphylococcus aureus, Streptococcus viridians, Pseudomonas aeuoroginosa, Staphylococcus epidermis, Candida albicans* and *Aspergillus tubingensis* against various antibiotics such as Gentamycin, Ampicilin, Tetracycline, Ciprofloxacin, Nystatin, Flucytosine, Amphotericin and Miconazole were studied. The antifungal activity of the Nystatin and Flucytosine were found to be nearly similar since the Nystatin is the drug of choice for *Candida albicans*. Efforts in prevention should therefore be redoubled in order to reduce the incidence of corneal ulcers and the burden they inflict on patients and on the healthcare system. The ubiquitous nature of *Pseudomonas aeruginosa* can be seen in its appearance in eyes with different ocular predisposing factors in this study. The predominance of Gram-negative bacilli in general and *Pseudomonas aeruginosa* in particular in contact lens-related ulcers in this study is corroborated in other studies. [9]

The similar studies reported that the antibacterial activity of Gram negative bacteria in general especially *Pseudomonas aeruginosa* in particular population indicates that the standard Ceftazidime and Gentamycin eyedrops used was appropriate, as no significant resistance to this antibiotic combination in the context of bacterial keratitis had been encountered local. [10] The results of previous study showed that the antimicrobial spectrum of defences against anaerobic bacterial strains showed that the most effective defend in against *B.vulgatus* and *B.fragilis* is HBD-3. In concentrations that kill *E. coli* and *S. aureus,* the constitutive (Human Beta-Defensins) HBD-1 exhibits in vitro no antimicrobial activity against *B. vulgatus*. Therefore, the described decreased induction of HBD-3 and the deficient

activity of HBD-1 may be responsible for a lower antimicrobial activity in Crohn's disease against *B.vulgates.* [11]

Lundstrom and Sobel (2004) reported that two antimicrobial agents have been approved specifically for the treatment of vancomycin-resistant enterococci (VRE) infections: quinupristin/dalfopristin and linezolid. [12] However, Quinupristin/Dalfopristin MIC90 results (16 mg/L) for *E. faecalis* systemic infections exceeds the maximum achievable serum concentrations, making this compound inactive for *E. faecalis*, and resistance among *E. faecium* has been recently increasing, especially in Europe among Vancomycin-resistant strains.

In our present study, we found that anterior chamber reaction associated Gram negative rods were more important compared to inflammation associated with Gram positive bacteria. This can be due to the higher pathogenicity of the Gram negative compared to Gram positive bacteria. Not surprisingly, the severity of the presenting signs (surface and depth of the infiltrate, presence of corneal new vessels and presence of anterior chamber inflammation) were significantly related to bacterial keratitis outcome.

CONCLUSION

The findings of the current study can be applied to the general population with microbial keratitis that prevails with a relative broad spectrum of severity as seen in a large ophthalmic centres, including primary, secondary, and tertiary patient care. However, caution is necessary in the application of the use of antibiotics as interpreted in this study. The available data concerning the biological control of these important keratitis pathogens were very limited, so the further experimental work on this area will be much great useful to the human society.

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