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Original Research Paper

Microbiology

Prevalence of microorganisms in Microbial Keratitis and their antibiotic and antifungal sensitivity pattern.

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ABSTRACT

Aim:Infectious keratitis is a vision threatening condition.If it is not properly diagnosed and treated with appropriate anti micribials, it will lead to loss of vision.The purpose of the study is to determine the prevalence of microorganisms & antibiotic and anti fungal sensitivity pattern of microorganisms involved in keratitis .Materials &Methods:From 120 patients with corneal ulcer a total of 75 organisms [bacteria & fungi] were islated & subjected for antibacterial and anti fungal sensitivity testing.Antibiotic sensitivity testing was carried out by disc diffution method by using antibiotics such as Gatifloxacin, Tobramycin,Ceftazidime,Vancomycin &Cotrimoxazole.Antifungal sensitivity pattern was carried out by disc diffusion method by using Amphotericin B,Itraconazole &Flucanazole. Results:All the bacterial isolates were 100% sensitive to Vancomycin .88% of S.pneumoniae spp,85% of Pseudomonas ,75% of Nocardia and 50% S.Viridans were sensitive to Gatifloxacin ,88% of S.Pneumoniae,and 71% of Pseudomonas ,50% of Nocardia and 50% of S.Viridans were sensitive to Tobramycin ,55.55% Of S.Pneumoniae ,42.8% of Pseudomonas 50% of Nocardia and 50% of S.Viridans were sensitive to Ceftazidime.Anti fungal sensitivity pattern of fungal isolates done by disc diffusion method showed that 71% Fusarium, 62% of A.Flavus,50% of A.fumigatus ,66.6% of A.niger,50% of Bipolaris were sensitive to Amphotericin B,82% of Fusarium ,75% of A.flavus,75% of A.fumigatus and 66.6% of A.niger and 50% of Bipolaris were sensitive to Itraconazole.All the organisms were resistant to flucanazole by disc diffustion method.Conclusion:90% of the bacterial isolates were sensitive to tobramycin followed by Gatifloxacin [88%] and Ceftazidime[55%]. All were sensitive to Vancomycin. Among the fungal isolates ,85% of them were sensitive to Amphotericin B and 90% of them were sensitive to Itraconazole.All the fungal isolates were resistant to Flucanazole by disc diffusion method.Knowledge of antimicrobial resistance is essential as it has prognostic value in the final clinical and visual outcome.

KEYWORDS :

Introduction: Infectious keratitis is a leading cause of corneal blindness in developing countries Corneal ulceration resultss in 1.5–2 million new cases of corneal blindness annually, posing a major public health problem according to the World Health Organization (WHO) reports An intact corneal epithelium acts as a barrier for the majority of microorganisms. Microorganisms can penetrate through a breach in the epithelium either due to penetrating or perforating ocular trauma or due to surgery.

Ocular morbidity such as corneal scarring and subsequent visual loss can be significantly reduced by prompt institution of appropriate therapy guided by the knowledge of the causative agents. An understanding of the clinical and microbial profile of corneal ulcers in a particular region helps us in improved management of this sight-threatening condition. The prevalence of blindness directly resulting from complications of suppurative keratitis is estimated to be 5%; the associated ocular morbidity is the result of several factors and patients management is directly affected by the lack of diagnostic facilities and initiation of appropriate antimicrobial therapy. Specific treatment requires quick and accurate identification of the causative microorganisms.

In conclusion, routine microbiological examination of patients with corneal ulcer is necessary in order to analyze and compare the changing trends of the etiology and their susceptibility patterns which would be beneficial in applying an appropriate antimicrobial treatment Timely use of appropriate antibiotics is therefore of utmost importance, especially in severe corneal ulcers4.

These antibiotics are empirically started while awaiting microbiological culture results. .Knowledge of antimicrobial resistance is essential as it has prognostic value in the final clinical and visual outcome. we restricted our study to aerobic bacterial and fungal agents and did not include anaerobic bacterial, amoebic and viral agents causing keratitis, complete analysis regarding the microbial profile was not possible3. Anaerobic organisms usually cause keratitis as mixed infection with aerobic organisms2. Not many studies have been conducted in this regard due to the cost and no feasibility of maintaining anaerobic culture methods.

Materials & Methods:This study was a prospective study & the study group comprised of 120 patients attending the cornea clinic at Department of Ophthalmology, Govt. Rajaji Hospital, Madurai (tertiary care hospital) and Aravind Eye Hospital(a private sector hospital dedicated to Ophthalmology), Madurai during the period from December 2010 to July 2011 The Institutional ethical committee clearance was obtained for study .

ANTIBACTERIAL SENSITIVITY TEST

Bacterial isolates were subjected to antibiotic sensitivity testing by the Kirby-Bauer's Disc Diffusion technique on Mueller Hinton agar plates as recommended by CLSI. Peptone water culture of the bacterial isolates corresponding to 0.5 McFarland's turbidity was used as inoculum.

The surface of Mueller-Hinton agar plate (after ensuring drying) was evenly swabbed in three different directions with a sterile cotton swab dipped into the inoculum Maximum six antibiotic discs were used for each 9 cm diameter plate. These plates were incubated at 370C for 16-18 hours in Ambient air. The diameters of zones of inhibition were interpreted according to CLSI standards for each organism. Media and discs were tested for quality control using standard strains.

The antibiotic discs used for bacterial isolates were Gatifloxacin, Tobramycin, Ceftazidime, Vancomycin and Cotrimoxazole.

ANTI FUNGAL SUSCEPTIBILITY TESTS

Antifungal susceptibility testing is receiving attention with the advent of newer anti fungal drugs. However susceptibility testing of filamentous fungi is not as advised as susceptibility testing. In vitro susceptibility tests should provide a reliable measure of relative activity of the antifungal agent, correlate with in vivo activity and predict the likely outcome of the therapy, provide a means with which to monitor the development of resistance and predict the therapeutic potentials of newer drugs.6 .

DISK DIFFUSION METHOD:

1. Inoculum preparation:

The fungal colony to be tested was grown in Potato dextrose agar slants at 35oc to induce the conidium and sporangiospore formation. After 7 to 10 days of incubation with well grown spores, the culture was taken for testing.

This method was performed on Nutrient agar or Muller Hinton agar

plates supplemented with 2% glucose.

The plate was allowed to dry for 10 minutes. Using a pair of flame sterilized forceps the antifungal disks were applied onto the surface of the inoculated plate. The plates were incubated at 35oc for 48 hours. The plates were read at 24 hrs and 48 hrs.

The following commercial Hi-Media antifungal disks were used:

Amphotericin B 100units	Itraconazole 10ug
Fluconazole 10 ug	Nystatin 100 units

RESULT

A total of 120 patients with infectious corneal ulcer were selected for study. This study involves males and females of all age group. 75 cases were culture positive.

Among the bacterial isolates, S.Pneumoniae 9/22 (40%) was the Predominant organism followed by Pseudomonas 7/22 (31%), Nocardia 4/22 (18%) and S.viridans 2/22(9.09%).

Among the fungal isolates, 28 out of 53 (52.83%) cases were due to Fusarium species and next common agent isolated was Aspergillus flavus 16/53 (30%), Aspergillus fumigatus 4/53 (7.5%), Aspergillus niger 3/53 (5.6%) and Bipolaris 2/53 (3.7%).

88% of S.Pneumoniae, 71% of Pseudomonas, 50% of Nocardia, and 50% of S.viridans were sensitive to Tobramycin. 55.55% of S.Pneumoniae, 42.85% of Pseudomonas, 50% of Nocardia, 50% of S.viridans were sensitive to ceftazidime. All the 4 species were sensitive to vancomycin and 22.2% of S.Pneumoniae, 42.8% of Pseudomonas, 50% of Nocardia, and 50% of S.viridans were sensitive to Cotrimoxazole.

Antifungal susceptibility pattern of fungal isolates by Disc diffusion method shown 62% of A. flavus, 50% of A.fumigatus, 66.6% of A. niger, 82% of Fusarium and 50% of Bipolaris were sensitive to Amphotericin B.

Antifungal susceptibility pattern of fungal isolates by Disc diffusion method shown 62% of A. flavus, 50% of A.fumigatus, 66.6% of A. niger, 82% of Fusarium and 50% of Bipolaris were sensitive to Amphotericin B.

Discussion

Antibacterial susceptibility pattern of bacterial isolates by Kirby-Bauer method showed that 88% S. pneumoniae spp, 85.7% of Pseudomonas, 75% of Nocardia and 50% of S.Viridans were sensitive to Gatifloxacin, 88% of S. pneumoniae and 71% of Pseudomonas, 50% of Nocardia and 50% of Strep Viridans were sensitive to Tobramycin. 55.55% of S.Pneumoniae, 42.8% of Pseudomonas, 50% of Nocardia and 50% Strep.Viridans were sensitive to Ceftazidime.

All the bacterial isolates were (Strep pneumoniae, Pseudomonas, Nocardia and Strep.Viridans) were 100% sensitive to Vancomycin.

This study is similar to the study of Cesar Espiritu et al9 in 2008 from Philippines which revealed 70% sensitivity of S.pneumoniae to Tobramycin, 84% sensitivity to fluroquinalone and 100% sensitivity to vancomycin.

Antifungal susceptibility pattern of fungal isolates done by disk diffusion method showed that 71% of Fusarium, 62% of A.flavus, 50% of A.fumigatus, 66.6% of A.niger, 50% of Bipolaris were sensitive to Amphotericin B. 82% of Fusarium, 75% of A. flavus, 75% of A. fumigatus and 66.6% of A.niger and 50% of Bipolaris were sensitive to Itraconazole.

All the organisms were resistant to Flucanazole by disc diffusion method.

The present study is similar to the study done by Usha Arora et al10 in 2006 from Amristar who reported that >81% of Aspergillus species were resistant to Flucanazole and Pankaj K Agarwal et al11 in 2001 from Calcutta whose study revealed that itraconazole is more effective in treating corneal ulcer. (more than 80% of fungi) were sensitive to itraconazole.

KL Therese et al12 in 2006 from Chennai has reported that A.niger exhibits high degree of resistance to Amphotericin B.

Summary

A variety of microbial organisms can produce infectious corneal ulceration.

Diagnostic corneal scraping and culture (Gold standard) are mandatory in order to identify the causative organisms when infective keratitis is suspected and to choose appropriate antimicrobial therapy.

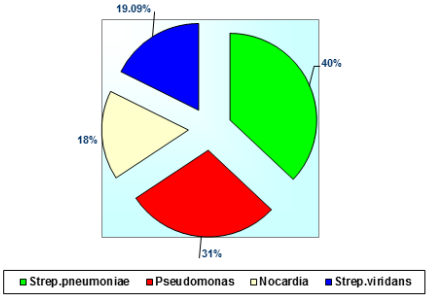
Among the bacteria, S. pneumoniae was the most common organism followed by Pseudomonas (31%), Nocardia (18%) and S. viridans (9.09%) 88% of the bacterial isolates were sensitive to Gatifloxacin, 90% of the isolates were sensitive to Tobramycin, 55% of the Isolates were sensitive to ceftazidime. All were 100% sensitive to Vancomycin.

85% of the fungal isolates were sensitive to Amphotericin B. 90% of them were sensitive to Itraconazole. All the fungal isolates were resistant to Flucanazole by disk diffusion method.

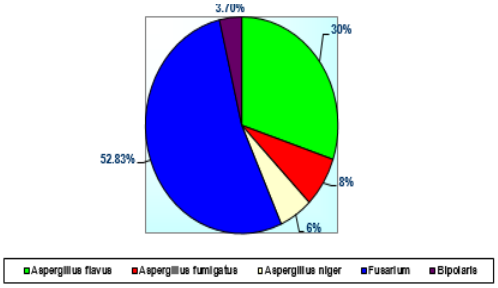
The present study indicates that the disc diffution method can be adopted for invitro antifungal sensitivity testing as it is simple, reproducible, cost effective and easy to perform technique in a routine clinical microbiology laboratory.

The increased incidence of fungal keratitis, coupled with a decreased bioavailability of donar corneas in developing countries, warrants further study of risk factors, antifungal susceptibility testing and possible pharmacologic combinations to prevent blindness.

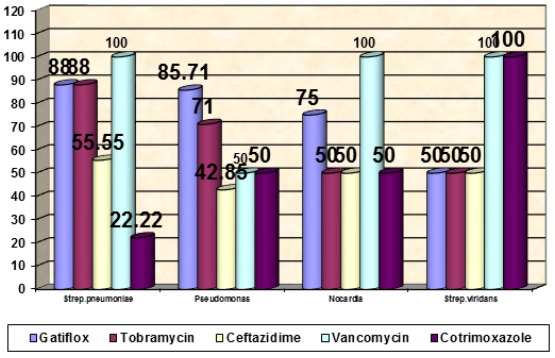
DISTRIBUTION OF BACTERIAL AGENTS CAUSING CORNEAL ULCER



DISTRIBUTION OF FUNGAL AGENTS CAUSING CORNEAL ULCER



ANTI BACTERIAL SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES



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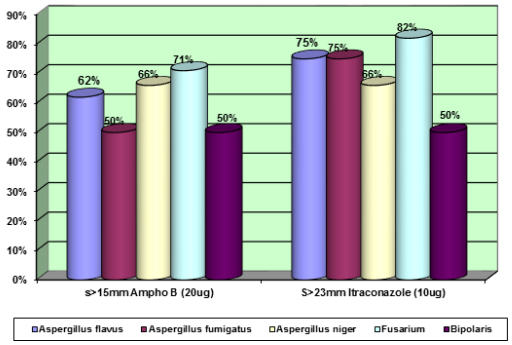

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