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Laboratory diagnosis and prevalence study of corneal infections from a tertiary eye care hospital

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ABSTRACT

To determine the prevalence of microorganisms involved in corneal keratitis and characterize the antibiotic sensitivity pattern. The 65 isolates screened were identified by Gram staining, Giemsa staining and acid fast staining (Ziehl– Neelsen) and cultured on different bacteriological media to identify the organism. The antibiotic susceptibility pattern was determined based on disk diffusion method using Mueller-Hinton agar with amikacin, gentamycin, erythromycin, ceftriaxone, nalidixic acid, ciprofloxacin, chloramphenicol, and cotrimoxazole antibiotic disks. Fungal isolates were identified with 10% Potassium hydroxide (KOH) and lactophenol cotton blue mount the scraped material was directly inoculated onto sabouraud's dextrose and potato dextrose agar plates. Staphylococcus aureus (33.47%) and Aspergillus spp (49.52%) were the predominant bacterial and fungal isolates few Acanthamoeba were also screened for keratitis. Agricultural practices as the main factor for corneal infections with high incidence in 31-40 age groups. Expect few S. epidermidis the organisms have gained drug resistance for frequently used chloramphenicol Pseudomonas spp and Klebsiella spp were found tetracycline and cotrimoxazole. Intermediate resistance was observed with amikacin, ceftriaxone, and nalidixic acid for most of the strains. Hence optimizing and management of infective keratitis will help to know the prevalence of the pathogens for appropriate microbiological testing and treatment.

Key words: Antibiotic resistance, bacterial infections, antibiogram, fungal mount.

INTRODUCTION

Corneal keratitis is a potentially sight threatening ocular condition and a leading cause of monocular blindness in developing countries like India [1]. Aetiologic and epidemiologic pattern of corneal ulceration varies with the patient population, geographic location and climate and it tends to vary widely [2]. Corneal ulcerations can be caused by different microbial agents like bacteria, fungi, virus and parasites. Although any organism can invade the corneal stroma if the corneal protective mechanisms such as blinking, tear dynamics and epithelial integrity are compromised, but microbial causes of suppurative corneal ulcers differs considerably in many regions [3]. Corneal diseases, especially infective corneal diseases, are major cause of blindness worldwide second only to cataract [4]. Among severe infective corneal ulcers, fungal keratitis is most common in many countries like China, India, Bangladesh, Nepal and the incidence is increasing in many countries [5, 6]. Most of the ulcerated eyes in these countries are treated empirically with topical antibacterial and antifungal agents; the spectrum of microbial agents associated with corneal ulcer is wide and varied. They arise as a consequence of contact lens wear, trauma, adnexal disease, topical steroid use, severe debilitation or ocular surface disorders and cause visual loss as a result of corneal scarring, perforation or endophthalmitis.

Bacteria causing corneal ulceration arise from a number of sources; the most common agents of bacterial keratitis are *Staphylococcus aureus*, *Streptococcus pneumoniae*, and gram-negative bacilli, especially *P. aeruginosa* [7]. Factors that have been correlated with this increasing incidence include the growing number of trauma cases, widespread abuse of broad-spectrum antibiotics and steroids. Hence understanding of the aetiologic agents, epidemiologic features and risk factors that occur in specific region are important in rapid recognition, timely institution of therapy, optimal management and prevention of disease entity.

The antibiotic resistant profile of gram negative non lactose fermenters are growing very rapidly in nosocomial settings than gram positive organisms [8]. These are common and prevalent even in environment and its associated systems [9, 10]. The purpose of this study was to identify causative pathogens and to determine the predisposing factors of corneal ulcer of patients attending tertiary care hospitals in Salem district, South India.

MATERIALS AND METHODS

A total of 65 clinically diagnosed patients of suppurative corneal ulcers of different age and sex who attended the Ophthalmology outpatient department (OPD) and also admitted in the Ophthalmology ward of Salem hospital during three months periods were included in this study.

Bacterial cultures and antibiogram pattern

Samples were obtained for bacteriological studies including direct smear and cultured on blood agar, chocolate agar, non-nutrient agar, thioglycolate, and brain-heart infusion broth. Blood agar plates were incubated under aerobic and anaerobic conditions, Chocolate agar was incubated with 5% carbon dioxide. The cultures were primarily identified by Gram staining, Giemsa staining and acid fast staining (Ziehl– Neelsen) [11]. The bacterial cultures were evaluated after 24 hours of incubation at 37ºC, if no growth occurred incubation continued for 48 hours. Study data including age, sex and drug history, previous incidence of ocular or systemic disease were recorded. Ten-fold serial dilutions of each inoculums were prepared in saline, 10^{-2} to 10^{-6} of diluents were pour plated on non-nutrient agar and allowed to stand for 10 minutes at room temperature. The numbers of organisms (colony forming units/ml) were determined for each dilution after 24 hours incubation at37°C; the isolates were inoculated on nutrient agar plates for further biochemical tests. The susceptibility of the organisms to the antibiotics amikacin, gentamycin, erythromycin, ceftriaxone, nalidixic acid, ciprofloxacin, chloramphenicol, and cotrimoxazole was performed based on disk diffusion method with Mueller-Hinton agar supplemented with 5% sheep blood and incubated for 18 h at 37°C as per NCCLS standards[12].

Screening for Fungal isolates

One corneal swab and three corneal scrapings were collected from each patient by an ophthalmologist with all aseptic precautions. Corneal swab was taken by rubbing the ulcerated area of the cornea with sterile cotton swab soaked with sterile normal. The material scraped was initially spread onto a labeled slide to prepare a 10% Potassium hydroxide (KOH) wet mount and lactophenol cotton blue mount. The second scraped material was directly inoculated onto sabouraud's dextrose and potato dextrose agar plates, incubated at 25°C to enhance the growth of fungi and observed daily for the first 7 days and on alternate days for the next 7 days, for observing slow growing fungi. Identification of fungal growth finally was done based on its macroscopic and microscopic features [13]. Only growth occurring on the "C" streaks was considered as significant and out growth away from the "C" streak was discarded as contaminants [14]. To screen for *Acanthamoeba* infection Non-nutrient agar seeded with *E. coli* was used.

RESULTS

Culture of corneal swabs and scrapings taken from 65 corneal ulcer patients yielded pure fungal growth, pure bacterial growth and mixed microbial growth. Of these (22.3%) had pure fungal growth, (77.7%) had pure bacterial growth and (17.2%) had mixed bacterial and fungal growth. Gram staining revealed 90.57% isolates of gram positive and 7.42% of gram-negative bacteria respectively. Further, culturing the ocular specimens on appropriate media and biochemical tests together confirmed the presence of various bacterial and fungal and no growth was observed in (14%) the dilution 10^{-2} was recorded with ≥109 colony forming units/ml.

Figure: 1 Distribution of bacterial and fungal isolates in corneal infection

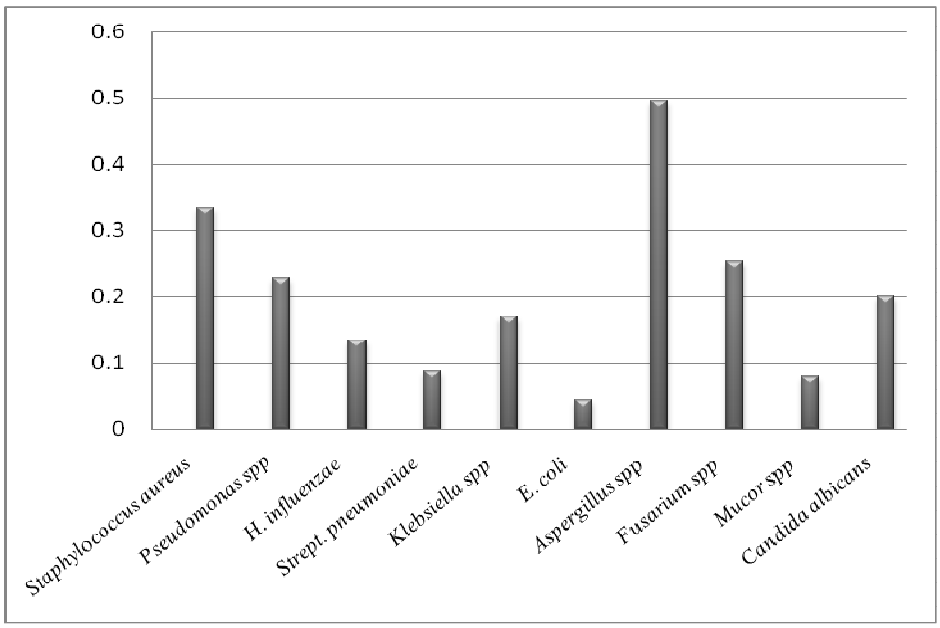

Figure: 2 Predisposed factors for keratitis

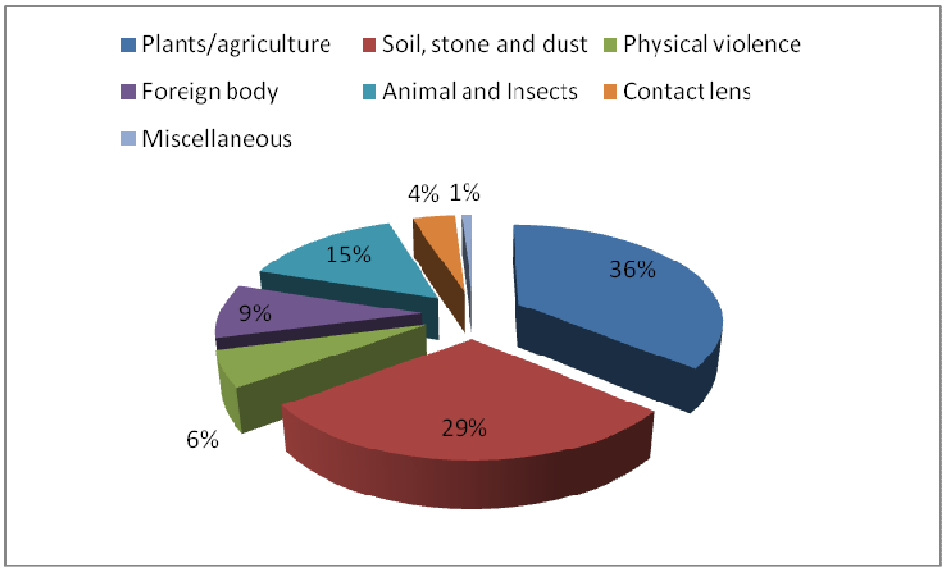

Table: 1Age and incidence of corneal infection isolates

Age(in years)	Total %of bacterial isolates	Total %of fungal isolates
< 10	8.57%	2.7%
11-20	14.28%	10%
20-30	20.00%	30%
31-40	31.42%	40.2%
41-50	8.57%	10.3%
> 50	17.14%	10.3%

Bacterial isolates (including both pure and mixed cultures) screened were *Staphylococcus aureus*, the leading bacterial pathogen representing (33.47%) completely resistant to erythromycin followed by *Pseudomonas spp.* (22.73%), *H. influenzae* (13.45 %), %), *S. pneumoniae* (8.69%), *Klebsiella spp* (17%) and *E. coli* (4.35%). Of the 65 patients with fungal keratitis, (60.2%) were male, and (39.2%) were female. *Aspergillus spp* (49.52%) were the most common fungal isolates followed by *Fusarium spp* (25.32%), *Mucor spp* (8%), *Candida albicans* (20%) and (1.0%) of *Acanthamoeba* keratitis. The male to female ratio was 1.6:1, the age of patients ranged from 7 to 82 years; patients of age groups 31-40 were most frequently infected. Both *Pseudomonas spp* and *Klebsiella spp* were found resistant to chloramphenicol, tetracycline and cotrimoxazole. Intermediate resistance was observed with amikacin, ceftriaxone, and nalidixic acid for most of the strains. Eighty-five percent of the infected patients were from agricultural background and most of them were infected during the harvesting season, the second common groups of patients were housewives with predominantly fungal keratitis.

DISCUSSION

The number of isolates collected correlated with previous reports [15]. On evaluating the correlation between direct microscopy and culturing, 80% positive from microscopic observation were unable to be cultured and only 42.3% yielded growth [1613]. The predominant isolate was *Staphylococcus* [17, 18], but *P. aeruginosa* was isolated commonly from corneal ulceration in contact lens users [19] and the distribution of other isolates are represented (Fig.1). In this study we found that due to frequent use of chloramphenicol the ophthalmic antibiotic, the organisms have gained drug resistance, expect few *S. epidermidis*. The occurrence and pathogenesis of Keratitis initially requires the adhesion of bacteria and initiation of infection starts by disrupting the corneal epithelium [20]. There are predisposing factors, namely, old age, operative manipulation and the excess use of topical corticosteriods and antibiotics for the onset of corneal infection in addition to other factors (Fig.2).The fungi are opportunistic organisms and colonize when the natural defenses of the eye are abrogated. Essentially, all are saprobic fungi and are not associated with infection in healthy individuals [21]. The main fungal pathogen was *Aspergillus* and *Fusarium* trauma is assumed to be an initiating factor although there is no clear incidence of injury [22]. Fungi reside as commensals in the flora of conjunctival sac in 3% to 28% of healthy eyes and may invade the cornea if the eyes are injured.

Corneal injury was identified as a major risk factor, with agricultural plants being the most common agents (25.7%) [23,24]. Severe infectious keratitis remains as a leading cause of ocular morbidity worldwide with potentially devastating visual impairment and significant costs to the public health system. Optimizing the prevention and management of microbial keratitis needs to be emphasized. Corneal infections are predominant in the age group 31-40(Table-1) [25]. There are many reports of plant products being used in the treatment of pathogens and infectious agents [26,27] Specific drug targeting systems are useful in precisely controlling the local spread of disease in many infection systems [28].

CONCLUSION

Continual education, both for ophthalmologists and patients, will minimize the incidence and severity of infection. Importantly in this study 80% of subjects admitted to hospital had at least one predisposing risk factor there is high prevalence of fungal infection reported unlike bacterial infections. An important factor in optimizing management of infective keratitis is to know the prevalence of the pathogens for appropriate microbiological testing and treatment. Despite advances in treatment, keratitis infection still remains clinically challenging and although the outcome can be favorable with appropriate management, there is potential for significant and permanent visual impairment in addition to social and healthcare costs.

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