Management of fungal corneal ulcer in rural areas of tropical countries.

M. Rezanur Rahman.

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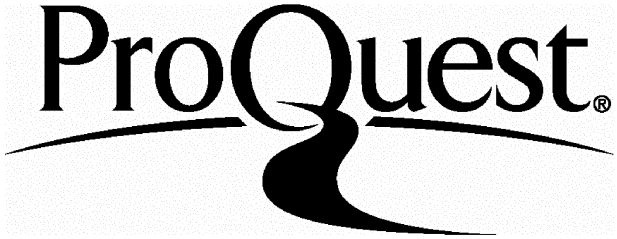
Institute of Ophthalmology,
University College London.



ABSTRACT

Suppurative corneal ulcer is a common problem in the tropical developing countries. A limited range of antibiotics is available but antifungal agents are usually completely unavailable there. Microbiological facilities are not available to identify the causal organisms. In addition ignorance, poverty, and illiteracy cause patients to use harmful traditional eye medicines, leading to blindness. In this study I have explored the use of chlorhexidine gluconate which should be cheap. In vitro I have tested fungal isolates from Ghana and India against chlorhexidine, povidone iodine, propamidine, polyhexamethylene biguanide (PHMB) and econazole by placing the drugs in wells in Sabouraud's dextrose agar media in petridishes. PHMB and propamidine showed no activity against the majority of fungi. Chlorhexidine showed a good dose related response. Econazole proved the most effective. In a pilot study chlorhexidine showed the best response compared with natamycin, povidone iodine and econazole. A masked randomised trial of three concentrations of chlorhexidine compared with 5% natamycin, showed that 0.2% chlorhexidine gave the best results without any toxicity. Another masked randomised trial in another country compared 0.2% chlorhexidine with 2.5% natamycin. 0.2% chlorhexidine showed the better response both in severe and non-severe ulcers. In vitro, I have tested chlorhexidine against different species of bacteria and showed good response both in Gram positive and Gram negative bacteria. Chlorhexidine has already been prescribed for the treatment of Acanthamoeba keratitis. It is also active against Chlamydia trachomatis. The method I applied here for fungal sensitivity testing is simple and cheap. Chlorhexidine is also cheap, stable in the tropical temperature and has already been used as an antiseptic and as a preservative for more than 40 years. I therefore suggest that chlorhexidine may be a useful first line agent in any suppurative corneal ulcer when microbiological facilities and antifungal agents are not available.

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1.0. INTRODUCTION : CORNEAL BLINDNESS

Corneal blindness is the second most important cause of blindness in the world after cataract. In contrast to the visual loss from cataract which affects mainly the older age group, corneal ulceration is encountered in all ages. It is the leading cause of blindness during childhood in most developing countries. About one-quarter to one-third of the estimated 38 million cases of blindness in the world are due to corneal damage[1].

Trachoma is still the most important cause of corneal blindness and may still account for 6 to 7 million cases of blindness in the world[1]. It is the second common cause of blindness next to cataract in Africa. In Mali it is the prime cause of blindness[11]. In north-west Kenya, trachoma and xerophthalmia are the major causes of blindness up to age of 35 and above the age of 45 it is cataract[9]. In the Northern Transvaal, trachoma is second cause of blindness accounting about 10% and women are mainly affected may be due to lower literacy[10]. However, not all these surveys distinguish between trachoma and other causes of corneal opacity. In those that do, causes other than trachoma may be very important. In Gambia non-trachomatous corneal opacity is more than the trachomatous opacity and accounting about 20% and 17% respectively[7]. In China corneal opacity due to trachoma varies from 2% to 26% while 7% to 30% is due to non-trachomatous corneal opacity[11].

About 30% of all blindness in some developing countries is caused by corneal opacity. It may be caused by a wide variety of species of bacteria or filamentous fungi. Sometimes corneal ulcers are caused by free living acanthamoebas. The most common bacteria are *Streptococcus pneumoniae*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis* and the most common fungi are *Fusarium* and *Aspergillus* species (Appendix-1). *Acanthamoeba polyphaga* keratitis was first identified in a South Texas rancher after corneal trauma in 1973[3], since then 68 cases were reported upto 1988, mostly in the United States[2]. A source of contamination can often be identified and an increasing number of case of Acanthamoeba keratitis have been associated with the wearing of soft contact lenses[5].

Minor trauma to the eye shortly before the onset of the keratitis was documented in most of the published reports. Hay, dust, sawdust, water and the use of contact lenses have been associated with the traumatic event[4]. A population-based survey of the prevalence of major causes of blindness and visual impairment was conducted in Bisha region, Saudi Arabia. Overall, 2882 people were examined and corneal opacity

which causes visual impairment is 3.8%. and the causes of corneal opacity is same for trachoma and other infections[13]. The other cases of corneal blindness in the world are due to corneal damage, Xerophthalmia, Trauma, Neonatal gonorrhoea, Leprosy, Onchocerciasis and harmful medical or non-medical eye practices. Most of the developing countries are in the tropical region. In the tropical countries about 30% of suppurative corneal ulcer is caused by fungus. On the other hand it is very difficult to tell the causative organisms by seeing the appearance of the corneal ulcer. The microbiological facilities are not available in the developing countries to identify the organisms. There are no facilities to do the antifungal susceptibility tests in the developing countries.

In the developing countries there is indiscriminate use of drugs, including even steroids as they are easily purchasable from any pharmacy and freely prescribed by village doctors. Antifungal drugs are not available and fungal keratitis is very difficult to treat. In Nepal, in spite of all available treatment the cause of unilateral blindness from corneal ulceration is 7.9% of all blind eyes[8]. Each year more than half of total number of eyes enucleated in the Eye Infirmary & Training Complex (EITC), Chittagong, Bangladesh come from patients with uncontrolled infection[46]. Keratitis due to filamentous fungi tends to occur in agricultural and out door workers. In developing countries agricultural trauma is the important risk factor for fungal keratitis. Most of the affected people live in rural areas where medical facilities are not available. The academic hospitals are situated in big cities and there continues to be a lack of ophthalmic microbiological facilities, ophthalmic trained nursing staff, equipment, beds and other trained personnel. In developing countries there are also logistic problems. Patients come with advanced stages of corneal ulcers so it is usually impossible to differentiate fungal or bacterial corneal ulcer clinically. The cost of microbiological services is very high. Due to lack of medical facilities, illiteracy, logistic problems and poverty, people are dependent on Ayurvedic medicines, traditional eye medicines, snail juice, rose-water etc. which leads to blindness.

2.0. THE AIMS AND OBJECTIVES

General objectives:

1. To prevent the blindness due to suppurative corneal ulcers.

Specific objectives:

1. To develop a simple and reliable method of culture of fungi which can be carried out in small hospitals and isolated situations in developing countries.
2. To develop a simple and reliable method of sensitivity testing against the antifungal agents which could reasonably be made available in developing countries.
3. To identify a single antimicrobial agent which is effective against bacteria, fungi and Acanthamoeba. The aim is that in any suppurative corneal ulcer it could be used when microbiological services are not available.

3.0. LITERATURE REVIEW

3.1. Previous reports of causes of microbial keratitis

Microbial keratitis results from a complex interaction between a wide array of pathogens and a diversity of host responses. The variability of these factors has hampered the development of simple management guidelines that will ensure an optimal outcome for most cases. The consequence of this has been the use of an extensive range of culture media in investigation, toxic levels of antibiotic treatment and constant clinical review.[38] The studies of fungus in the different countries and latitude show that the spectrum of micro-organism responsible for suppurative corneal ulcer varies according to geographic location (Appendix-1). So the understanding of distribution and causative organisms of corneal ulcer all over the world is essential to prevent blindness from corneal ulcer.

Fungal corneal ulcer in tropical and sub-tropical regions is predominantly caused by filamentous fungi rather than yeast species. In tropical regions environmental factors such as humidity, rain fall, temperature, and wind accounts for fungal keratitis[46,47]. In South Florida the incidence of fungal keratitis increases in the month of November when the climate becomes dry and windy[47]. Wind speed was believed to stimulate frictional or mechanical turbulence, causing eddying and thereby aiding the upward and lateral dispersion of fungal spores. In one study from Florida there was no fungal keratitis in contact lens wearers[51]. The most common organism responsible for fungal keratitis on a world wide basis is *Aspergillus sp*[48]. *Aspergillus* is the important causal organism of fungal keratitis in Bangladesh, Nepal and India. But in Aravind eye hospital, Madurai, India, fungal keratitis is 50% and most important causal organism is *Fusarium* (personal communication). *Fusarium sp.* have been isolated from cornea in cases of fungal keratitis through out the world including north, central and south America, Europe, Africa, Middle East, India, China, Japan and Bangladesh[48]. But *Fusarium sp.* is the most important cause of fungal keratitis in South Florida. In northern United States *Candida sp.* and *Aspergillus sp.* are isolated more frequently in fungal keratitis where as *Fusarium sp.* is the major etiologic agent in the Southern United States[48]. The causal organisms are also changing in the same location and new fungi are isolated. In South Florida *Fusarium solani* was the commonly isolated organism between 1959 & 1977, but *Fusarium oxysporium* being the most commonly isolated organism between 1982 & 1992[48]. In South Florida 4 new fungi include

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Candida parapsilosis, *Candida tropicalis*, *Aspergillus terreus*, and *Trichosporon begellii* were isolated from fungal keratitis[48]. In Bangladesh a new fungus *Dichotomophthoropsis nymphaerum* has been isolated[49]. In fact more and more fungus are isolated especially from tropical regions and the number of species of fungus is more in tropical regions than the other parts of the world. It is seen that the highest incidence of fungal keratitis is in Bangladesh and India but the number of causal organisms isolated are more in South Florida and Nepal.

It is also seen that closer to the equator the more the number of species and more away from the equator the less the number of species. In Bangladesh and India more species are yet to be identified. Some of the underlying factors for this large incidence of corneal ulcer in Bangladesh may be due to vitamin and protein deficiencies. The percentage of fungal corneal ulcer is more to the equator than away from equator and all the countries studied here are situated north to the equator except South Africa. As South Africa is 26° south of the equator and at a high altitude the fungal keratitis may be less there. A brief outline of the distribution of corneal ulcer is in Table 1 and Graph 1.

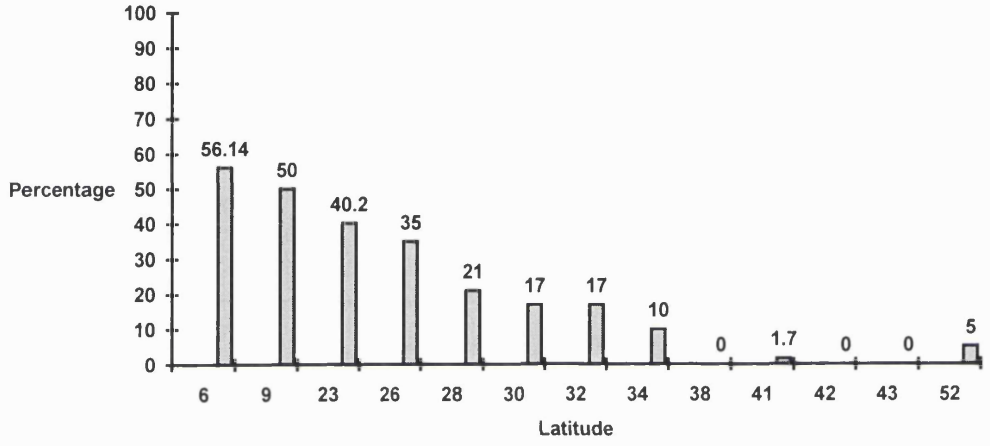
Table no. 1

Geographical distribution of fungi.

Sl.no.	Geographical location	Fungus	Latitude	No. of species.	References
1.	Ghana (Accra)	56.14%	6° (North)	17	41
2.	India (Madurai)	50%	9° (North)	7	14
3.	India (Trichurapalli)	30%	11° (North)	4	54
4.	Bangladesh (Chittagong)	40.2%	23° (North)	14	46
5.	South Florida (Miami)	35%	26° (North)	30	47, 12
6.	South Africa (Soweto)	7%	26° (South)	8	56
7.	Nepal (Kathmandu)	21%	28° (North)	34	8
8.	Houston Jones	17%	30° (North)		52
9.	Texas	17%	32° (North)	-	12
10.	California (Los Angeles)	10%	34° (North)		52
11.	San Francisco	0%	38° (North)		52
12.	New York	1.7%	41° (North)	4	12
13.	Boston	0%	42° (North)		52
14	Toronto, (Canada.)	0%	43° (North)		6
15.	London	5%	52° (North)	2	57

Graph-1

Fungi as a percentage of suppurative keratitis in relation to latitude



In the last three decades the number of reported cases of fungal keratitis has increased dramatically possibly due to increased awareness of the disease, advances in laboratory diagnosis and increased use of topical steroids and broad spectrum antibiotics[48]. Corticosteroids are believed to predispose to mycotic keratitis by suppressing ocular immune mechanisms. They also seem to encourage the growth of fungal opportunists. Corticosteroids may activate non-pathogenic fungi and increase virulence of pathogenic fungi[48]. In South Florida each year about 45% cases of suspected infectious keratitis are culture negative due to partially treated bacterial and fungal keratitis[47], otherwise the percentage of fungal keratitis might be higher.

Effective treatment of corneal ulcer depends on early recognition of responsible pathogens. However we do not necessarily require to identify the specific organism. What we need is to differentiate bacteria, fungus and acanthamoeba. Then we need to develop a simple method to culture the organisms and to see the sensitivity against 2 to 3 antifungal agents which could be made available in that country. These methods should be very simple so that local ophthalmologists and general physicians will have practical methods of controlling fungal corneal ulcer in rural areas. At the same time it should be cheap so that every body may be benefited.

3.2. Ophthalmic antibiotics

A. Penicillins.

Mechanisms of action.

They interfere with the biosynthesis of the peptidoglycan structure of bacterial cell walls; cause a decrease of murein hydrolase, which then permits autolysis to occur; and have effects on the intracellular enzyme system[53].

Naturally occurring penicillins

1. Aqueous crystalline penicillin G
2. Procaine penicillin G
3. Benzathine penicillin G

Semisynthetic Penicillins

1. Ampicillin
2. Amoxicillin
3. Hetacillin
4. Carbenicillin Indanyl
5. Methicillin
6. Carbenicillin

Methicillin

It is the preferred semisynthetic penicillin for use against unidentified gram-positive infections. Although naficillin, oxacillin and cloxacillin are more active by weight than methicillin is against penicillinase-producing *staphylococci*, these differences are probably insignificant because of adjusted dosages and degree of serum binding[50]. Protein binding theoretically reduces the amount of free antibiotic available and restricts its distribution in the extravascular space. The in vitro minimal inhibitory concentration of methicillin is not altered in serum, as to an eight fold or greater increase of the other penicillinase-resistant penicillins. It is reasonable to propose that the proteinaceous exudate and the stromal infiltration of corneal ulceration bind antibiotics and thereby reduce the amount of available free agent. All of the semisynthetic penicillins are less effective by weight than penicillin G against non-

penicillinase-producing *Staphylococci*, diplococci and *Streptococci*. Methicillin has the same side effects associated with the other natural and semisynthetic penicillins and may also produce a specific form of nephropathy.

Carbenicillin

It is the first semisynthetic penicillin effective against *Pseudomonas* and *Proteus*. Its bactericidal activity is exerted by inhibition of the cell wall synthesis of sensitive organisms and inducement of the formation of spheroblasts[50]. It is less effective than penicillin G against sensitive gram-positive cocci and is not active against penicillinase-producing *Staphylococci*. It shares the toxic and allergic reactions of the penicillins and may induce haemorrhagic phenomena within the gastrointestinal tract and urinary bladder.

B. Aminoglycosides

Mechanisms of action

This disrupts the cycle of ribosomal function by interfering with the first step of protein synthesis. They also induce misreading of the genetic code of messenger RNA template, incorporating incorrect amino acids into growing polypeptide chains[53].

1. Tobramicin.
2. Streptomycin
3. Neomycin
4. Kanamycin
5. Amikacin
6. Gentamycin

Gentamicin

On the basis of its bactericidal spectrum, stability, intraocular penetration, efficacy and low dose toxicity, gentamicin is the preferred antibiotic for use against unidentified Gram-negative infections. Its bactericidal activity is accomplished by inhibition of bacterial protein synthesis. It is effective against 90 percent or more of strains of *Pseudomonas, Aerobacter, Klebsiella, E. coli, Serratia marcescens* and other Enterobacteriaceae[50]. Moraxella species are variably sensitive. In vitro it is active against penicillinase and non-penicillinase-producing *Staphylococci* and has been

clinically effective in serious staphylococcal infection. Gentamicin shares some of the toxic effects of the other aminoglycosides and becomes ototoxic if serum levels exceed 10 μg per millilitre.

C. Fluoroquinolones

1. Norfloxacin
2. Ofloxacin
3. Ciprofloxacin

They are less toxic than aminoglycosides and are all available as 0.3% solutions. Clinical trials of these in keratitis are only available for ciprofloxacin 0.3% and have shown similar or better results than for conventional therapy. In vitro antimicrobial sensitivity studies show that norfloxacin is less active against Gram-positive isolates than either of the other two quinolones for non-ocular isolates and this has been confirmed for ocular isolates in comparison with ofloxacin[38]. For these reasons norfloxacin is the least appropriate of these available quinolones as broad spectrum monotherapy for bacterial keratitis. Either ciprofloxacin or ofloxacin should be effective as monotherapy for most cases of bacterial keratitis. Ofloxacin penetrates the cornea better than other commercially available fluoroquinolones and human tear film concentrations of ofloxacin, 4 hours after topical administration, exceed the MIC for a wide range of ocular isolates. Ofloxacin, unlike ciprofloxacin, does not induce corneal plaques. These data suggest that ofloxacin should be as effective as ciprofloxacin for bacterial keratitis.

D. Polypeptides

1. Gramicidins
2. Polymyxin B and E
3. Bacitracin

Bacitracin

Although the penicillins may be applied topically, bacitracin is an effective substitute and may minimise the risk of penicillin sensitisation. This agent achieves its bactericidal activity by binding to cell membranes to produce false pores and flux of ions. It is effective against Gram-positive cocci, including penicillinase-producing

Staphylococci and *Neisseria*[50]. Allergic sensitisation or primary irritation following topical application is rare. Nephrotoxicity prevents parenteral administration.

E. Cephalosporins

Mechanisms of action

They inhibit a transpeptidase completely, thereby preventing a cross-linkage of new murein sub-units. This action results in a structurally weakened cell wall marked by accumulation of cytoplasm and rupture of the cell wall, caused by increased osmotic pressure[53].

First-generation Cephalosporins

1. Cephalothin
2. Cephaloridine
3. Cephalexin
4. Cefazolin
5. Ceftezole
6. Cephradine

Second-generation cephalosporins

1. Cefamandole
2. Cefoxitin
3. Cefaclor
4. Cefuroxime

Cefuroxime

It is a second generation cephalosporin. The cefuroxime levels in primary aqueous humour of humans, when measured between 30 minutes and 6 hours, are higher after I.V. than I.M. administration[53]. These levels are significantly greater than the MICs of most ocular pathogens. Its poor antibacterial activity against pseudomonas, however, necessitates a combination treatment for severe infections, when no causative organisms can be detected.

Third-generation cephalosporins

1. Cefotaxime
2. Ceftizoxime
3. Cefoperazone
4. Moxalactam
5. Ceftazidime

F. Tetracyclines

They are bacteriostatic and induce inhibition by interfering with the protein synthesis of bacteria. They are the drug of choice for chlamydial infection, including inclusion conjunctivitis, trachoma, lymphogranuloma venereum and psittacosis[53]. They are also the drug of choice for blepharitis and conjunctivitis caused by susceptible organisms. Recently, minocycline has been shown to be effective against experimental ocular toxoplasmosis.[53]

G. Macrolide and related

1. Erythromycin
2. Clindamycin
3. Lincomycin
4. Vancomycin
5. Chloramphenicol

Chloramphenicol

Chloramphenicol exerts its bacteriostatic activity through the inhibition of protein synthesis. Most Gram-positive and Gram-negative organisms are susceptible, including N. gonorrhoeae. It is effective against Mycoplasma, rickettsiae, Chlamydia and spirochetes[53]. Topical administration of chloramphenicol 0.5% solution has been shown to achieve effective antibacterial levels in the aqueous and cornea.

H. Sulfonamides and sulfones

The action is bacteriostatic. They act by competing with extracellular para aminobenzoic acid (PABA), a substance that susceptible organisms require to form folic acid. Folic acid is essential for the production of purines and to the ultimate

formation of the nucleic acid. They have been used in trachoma, gonococcal ophthalmia, streptococcal membranous keratoconjunctivitis, blepharoconjunctivitis and acute dacryocystitis[53].

3.3. Antifungal drugs

1. Polyenes:

Amphotericin-B- either fungistatic or fungicidal
Nystatin-both fungistatic and fungicidal.
Natamycin (Pimaricin)-fungicidal
Etruscomycin (Tetraene).

2. Azoles:

A.Imidazoles: (Two Nitrogen atoms)

Clotrimazole-fungicidal.
Econazole
Ketoconazole
Miconazole-fungicidal.
Isoconazole
Sulconazole
Tioconazole
Oxiconazole
Thiabendazole

B.Triazoles: (Three Nitrogen atoms)

Fluconazole
Itraconazole
Saperconazole

3.Pyrimidines:

Flucytosine (Fluorinated) or 5-fluorocytosine-fungistatic.

4.Diamidines:-Fungistatic

Propamidine
Dibromopropamidine
Hexamidine
Iodohexamidine
Hydroxystilbamidine Isethionate

5. Chlorhexidine:- Fungicidal.

6. Povidone-Iodine:- Fungicidal.

7. Silver sulphadiazine.

8. Aureofuscin

9. Fatty acids and their salts:

Proprionates

Undecylenic acid

10. Tolnaftate

11.Haloprogin

12.Benzoic acid and salicylic acid.

13Miscellaneous:

Griseofulvin-fungistatic.

Terbinafine

Acrisorcin

Candicidin

Chlordantoin

Carbol-fuschin

Sulphur and Thiosulphates

Iodoquinol (diiodohydroxyquin)

Clioquinol (iodochlorohydroxyquin).

The available antifungal drugs reach fungistatic but rarely fungicidal, levels in the tissue. The antifungal drugs which are fungicidal show fungistatic action when applied to the cornea because low concentration of drugs are used on cornea while in high concentration they shows fungicidal property. The objective of antifungal therapy is to inhibit fungal growth over a long period so that the body's defence mechanism can manage the fungus[30]. Fungal infections in industrialised countries are frequently associated with a defect in host resistance which should , if possible, be corrected otherwise drug therapy may fail.

Mode of action:

Polyenes:- (Nystatin, Amphotericin, Natamycin).They combine with the sterol of cell membranes and result in an increase in the permeability of the membranes allowing leakage of small molecules.
Imidazoles:-(miconazole,econazole, clotrimazole), permeate the chitin of the fungal cell wall and increases the membrane permeability to various intracellular substances.

Diamidines:-(propamidine, dibromopropamidine, hexamidine, iodohexamidine)
Inhibit the oxidative metabolism of bacteria.

Antifungal drugs for ocular use are usually not commercially available. The following are used in Moorfields Eye Hospital which is one of the specialist reference centres for the United Kingdom.[32,27]

Routes:

1. Intravitreal
2. Subconjunctival
3. Systemic
4. Topical

Systemic route:

1. Flucytosine
2. Ketoconazole
3. Itraconazole
4. Amphotericin-B (toxic)

Topical route:

1.Amphotericin-1/2 or 1 hour interval and reduce doses as condition improves continue may be month. Normally active against *Candida.*
2. Miconazole-same doses
Active against *Candida* and Gram positive bacteria.
3. Clotrimazole-same doses
Active against *Aspergillus, Candida* and *Acanthamoeba.*

4. Econazole-same dose
Active against *Fusarium, Aspergillus, Penicillium.*
5. Natamycin 5% drop-same dose
Active against *Fusarium, Aspergillus, Candida.*

Amphotericin-B.

No intraocular penetration except by direct intraocular injection. Because of poor penetration, it cures only superficial infections when applied topically[30]. It is reported that 0.15% solution of amphotericin B is non-toxic and non-irritant to the eye[54]. Intravenous injection should start with 250 microgram/kg daily, gradually increasing the dose if tolerated to 1 mg/kg daily, maximum 1.5 mg/kg daily or on alternate days[27]. Amphotericin-B is not absorbed from the gut and is the only polyene antibiotic which can be given parenterally[27]. The antibiotic is either fungistatic or fungicidal, depending on the concentration of the drug and the sensitivity of the fungus and it is without effect on bacteria, rickettsiae or viruses[15].

Nystatin

It is not absorbed from the gut but is too toxic for parentral use[27]. Nystatin is reasonably well tolerated in the eye as a 3.3% ointment. It has a medium level of activity against most *Candida* isolates and an occasional isolate of the other ocular fungal pathogens and is active against superficial infections[30].

Natamycin.

A 5% ophthalmic preparation is commercially available. It has got a wide range of antifungal activity against ocular pathogens. It is the least irritating and least toxic of the Polyene antifungals[30]. It can be used as a first line of treatment in any suspected fungal infection. It is relatively stable and can be sterilised by heat without loss of potency. It can certainly cure many superficial fungal infections but is ineffective against deeper infections in the cornea[30].

Clotrimazole

It has got a broad spectrum antifungal activity with low human toxicity. Topically it is as good as the Polyenes. In severe infections it is used both topical and oral administration of 60 mg/Kg/day to 100 mg/Kg/day. In children 150 mg/kg/day[30]. It

can be given to patients with renal dysfunction induced by Amphotericin-B but should not be given in the first three month of pregnancy and to those with severe liver or adrenal disease[30]. It is active against *Aspergillus sp*[28]. infection. Clotrimazole 1% solution in arachis oil is recommended[32].

Miconazole.

This is a broad spectrum antifungal with low mammalian toxicity[30]. It is as active as Benzylpenicillin against Gram positive bacilli and cocci. It is not significantly absorbed when applied topically but is partially absorbed at oral administration. A daily oral dose of 0.3 Gram is well tolerated. It does not induce drug metabolising enzymes in liver. In severe infections it can be given intravenously. Topical 1% solution is as effective as Nystatin. It is active against *Candida sp*[30].Topical use of this compound is sometimes associated with superficial punctate keratitis[34].

Econazole.

This is a broad spectrum antifungal, which does not induce drug-metabolising enzymes in the liver. The oral dose is 0.3 Gram/day. Topical 1% solution is active against *Fusarium sp., Aspergillus sp.* and *Penicillium*[30]. It is less irritating and can be given intravenously in severe infection. Jones and associates considered econazole to be most widely acting drug for the treatment of mycotic keratitis when compared to other azoles[34].

Thiabendazole

It is an antihelmintic. It penetrates the eye well in a 4% suspension and is non-irritating to the eye. It is particularly active against some ocular isolates of *Fusarium* species and other filamentous fungi including *Penicillium, Phialophora* and *Cladosporium* species[30]. It can be given orally to back up intraocular penetration from topical administration.

Ketoconazole

The dose is 600mg/day orally and topical 1% suspension. Oral administration may produce effective drug levels in the cornea and aqueous[31]. Combined topical and oral ketoconazole was very effective in treating mycotic keratitis but was ineffective in *Aspergillus fumigatus* keratitis with intraocular invasion. Oral therapy may be

associated with a transient rise in certain serum enzymes and topical therapy may result in transient superficial punctate keratitis[34].

Itraconazole

Oral therapy brought excellent responsiveness with mycotic keratitis. It is the drug of choice for treating keratitis due to *Aspergillus spp*[34]. Oral as well as topical therapy could be administered with minimal or no side effects. 1% suspension for topical use has been prepared and is generally well tolerated.

Saperconazole

A triazole derivative. It exhibits broad spectrum activity against many fungi, especially *Aspergillus sp.* and certain dematiaceous fungi and was found active following topical, oral and parentral administration[34].

Fluconazole

A triazole derivative. It penetrates rabbit eyes better than ketoconazole and itraconazole. Topical therapy was found to be useful for keratitis due to *Candida sp*[34]. Oral fluconazole produces effective drug levels in the cornea, aqueous, vitreous and choroid/retina in animals[31].

Flucytosine.

It is well absorbed orally and is remarkably non-toxic in human beings because it is not metabolised but excreted unchanged in urine[30]. It has got a synergistic effect on *Candida* with Amphotericin-B. Oral dose is 200mg/kg/day. Topical 1.5% drops are non-irritating to the eye. Sub-conjunctival injection is disappointing.

Aureofuscin

This is an antifungal agent from China which was used topically as a 0.1% solution or a 1% ointment to treat mycotic keratitis, and a success of more than 80% was reported[34].

3.4. Non-specific antiseptics

Propamidine (Brolene)

Gram positive species are all more susceptible than Gram negative organisms[25]. The unionised propamidine penetrates bacterial cell wall[26]. Dibromopropamidine and iodohexamidine were found to be the most effective of the compounds[25]. It is effective in persistent angular conjunctivitis caused by *Morax-Axenfeld bacillus*[24]. Fungistatic activity was determined by noting the highest dilution which caused complete inhibition of growth of fungus after five days at room temperature[21].

Chlorhexidine.

It is used as topical antiseptic for application to skin, wounds and mucous membrane and for dental use. It has been used as pharmaceutical preservative, particularly ophthalmic solutions and as a disinfectant for items such as inanimate surfaces and instruments[16]. It disrupts the plasma membrane of the bacterial cell. It is poorly absorbed from the gastrointestinal tract and negligibly absorbed from the skin of adults.

Antimicrobial action: The antimicrobial activity is directed mainly toward vegetative Gram-positive and Gram-negative bacteria. It is inactive against bacterial spores except at elevated temperatures. At low concentrations it is bacteriostatic whereas at high concentration it is bacteriocidal[16]. The in vitro bactericidal and fungicidal activity of 0.05% chlorhexidine gluconate was determined using a procedure based on British Standard 3286 (1960)[16]. Acid-fast bacilli are inhibited but not killed[36]. It is active against some lipophilic viruses e.g. influenza virus, herpes virus, HIV. Fungicidal activity in general is subject to species variation. It also has an effect against Chlamydia[23]. In high and low concentrations it effectively kills cysts of Acanthamoeba spp.[36,79].

Wound healing: It slightly delays wound healing but using in infected wound it actually accelerates the rate of healing.

Skin irritation and sensitisation: It is not skin irritant with any concentration.

Oncogenicity: It is not a carcinogen.

Effect of pH: For antimicrobial activity the optimum range is 5.5 to 7.0.

Storage: Chlorhexidine solution may be stored at room temperature for at least 1 year.

Sterilisation: A solution of less than 1% of chlorhexidine may be autoclaved at 115^0C for 30 minutes or for 15 minutes at 121^0C to 123^0C. A concentration of more than 1% causes insoluble residue.

Solubility: Chlorhexidine digluconate is very soluble but chlorhexidine diacetate is soluble at 1.9% W/V.

Toxicity

A. Cornea.

Animals:

1. Aqueous solutions containing 2%, 1%, and 0.1% chlorhexidine were given one drop in rabbit eyes twice daily for one to seven days. Daily examination of the rabbit eyes receiving the chlorhexidine solutions revealed no gross or microscopical changes. Rabbit corneas treated with a 2% solution of chlorhexidine, two drops four times a day for one week, revealed no changes. Except for its effect of binding mucous to the surface of the lenses, chlorhexidine in the concentrations used, is an ideal bacteriostat. It has the following properties: l. chemical compatibility with ophthalmic drugs, ll. wide bacteriostatic and bactericidal activity against those organisms likely to be encountered in both the compounding and use of the solution, lll. solubility in water and buffer solutions, lV. no effect on the pH and tonicity, V. non-toxic and non-irritant to the ocular tissues, Vl. stable after prolonged storage, Vll. heat-stable (permitting autoclaving)[35].
2. In the subacute direct instillation studies in rabbits , a slight circumcorneal injection and conjunctivitis were observed in both experimental and control eyes. A concentration-dependent increase in the incidence of these reactions was noted as the chlorhexidine digluconate content increased from 0.005% to 0.05%. No deleterious ocular responses were observed throughout the gel lens wearing studies. These studies suggest that chlorhexidine digluconate formulated properly has merit as a gel lens sterilising agent[43].
3. Following direct topical application of up to 2% chlorhexidine in rabbits eye, no changes to the cornea were seen by direct observation or light microscopy; however,

superficial epithelial changes were noted by electron microscopy following application of 0.1% and 0.5% chlorhexidine[16].

4. Concentrations of greater than 2% were clearly toxic to both the corneal epithelium and conjunctiva: a concentration of 1% produced no significant delay in epithelial healing but did cause mild conjunctivitis. Concentrations less than 1% were not statistically different from the control group either in re-epithelialisation or visible toxic effects[16].

5. Irrigation of rabbits cornea with 2% and 4% chlorhexidine significantly slowed the healing rate compared with saline control. Irrigant concentrations of ≤ 1% did not statistically delay healing. Topical aqueous chlorhexidine may be an alternate agent for preoperative cojunctival antisepsis[59].

6. Hibiclens (chlorhexidine 4% and detergent) exposed to rabbit eyes for varying time intervals ranging from 5 to 15 minutes causes severe, irreversible and progressive corneal damage[60].The effect of the detergent was not distinguished from that due to the chlorhexidine.

Human

7. Occasionally chlorhexidine may cause irritation of corneal epithelium, although it may depend on the formulation used[61].

B. Nervous system

Animals

8. Chlorhexidine caused a marked and dose-dependent degeneration of adrenergic nerves when injected into the anterior chamber of albino rat eye. Two days after the injection of lowest dose of 0.05% chlorhexidine, approximately 30% of the nerves had disappeared. Almost complete degeneration was observed after the same time with higher doses of 0.5%, 1% and 1.5% chlorhexidine. Two weeks after the lowest dose, the nerves had regenerated almost completely. With the highest dose used, only some 40% of the normal adrenergic nerve plexus had reformed after 51 days. This suggest that neurotoxic actions on thin unmyelinated fibre systems should be looked to also in the central nervous system[62].

9. Dose-dependent inhibitory effect of chlorhexidine ($2.5x10^{-5}$-$5.0x10^{-4}$) g ml. on neuromuscular transmission were localised by tension and electromyogram recording during indirect stimulation on the isolated rat phrenic nerve-diaphragm preparation[63].

Human

10. Chlorhexidine has been widely used in medical practice since early 1950s. This extensive experience has demonstrated the virtual absence of sensitisation and a low irritancy potential for the compound. Only one significant adverse effect has been identified during medical use. This is sensorineural deafness after direct instillation of commonly used chlorhexidine into the middle ear cavity[64].
11. Chlorhexidine 1% gel does not affect the adrenergic innervation of buccal mucosa[65].

C. Oral mucosa

Animal

12. There were no statistically significant differences in enzyme activity between the placebo group and individuals who used 0.2% chlorhexidine for 18 months[77].
13. Chlorhexidine (Hibitane) is poorly absorbed after oral administration, and its percutaneous absorption is absolutely minimal. No clinical or histological effects have been obtained in any animal study to cause hesitation in the light of proliferating applications of chlorhexidine in human use[66].
14. Chlorhexidine cause bone loss during the treatment of experimentally induced active periodontitis in dogs[67].

Human

15. Chlorhexidine treatment reduces plaque and gingivitis, but tended to stain teeth when used as 10 ml. of 0.2% aqueous solution of chlorhexidine gluconate daily in addition to tooth brushing and interdental cleansing. Stains were readily removed by a conventional dental prophylaxis procedure. There were no other local side effects relative to the structure and function of the oral mucosa, tongue, salivary glands and pharyngeal complex following the prolonged use of chlorhexidine[68].
16. There was no changes in the normal structure of keratinizing oral epithelia as a result of prolonged daily exposure to 0.2% chlorhexidine for one year[72].
17. During two years of daily use of chlorhexidine mouth rinses, no systemic or local side effects were observed. Chlorhexidine is poorly absorbed following oral dosing and that the major excretory route is in faeces[73].

18. Very occasionally a reversible swelling of the parotid glands has been reported after use of chlorhexidine in mouth rinse formulations. Occasional oral intolerance of mouth rinse formulations has been reported, although no histological abnormalities were present in gingival biopsies taken after 18 months' daily use[64].
19. After periodontal flap surgery 1% chlorhexidine gel effectively reduces pain and swelling compared to penicillin. Chlorhexidine may be a suitable alternative to penicillin after periodontal flap surgery[74].
20. Allergy is extremely rare. Occasionally there may be parotitis or sore mouth. The most common problems are staining of the tongue and the teeth, interference with taste and an unpleasant taste of the compound as prepared. All these effects are rapidly reversible[61].
21. Intensive treatment with chlorhexidine gel, in individually fitted custom trays, combined with meticulous oral hygiene measures may induce toxic effects on the surface layers of the gingiva and mucosa[70].
22. Skin and oral tissue cells in culture were exposed for an hour to 0.005% and 0.002% chlorhexidine and for 30 seconds to 0.12% chlorhexidine. 0.002% showed minimal cytotoxicity but is able to suppress cell division almost completely. Chlorhexidine is highly cytotoxic to cells in vitro, but various cell functions such as proliferation, collagen gel contraction and protein synthesis are affected to different degrees by the drug[69].
23. In Norway, chlorhexidine has been dispensed for over 20 years. But the only side effects were staining of teeth and tongue and dryness of mouth, and occasionally oral ulcerations. Only 4% of the dentists recommended mouth rinse with 0.1% chlorhexidine, whereas 96% recommended 0.2% chlorhexidine[71].

D. Other systems

Animals

24. The effect of 0.05% chlorhexidine in chronic ulcer healing did not differ from saline[75].
25. Chlorhexidine was used as wound irrigation fluids upon femoral arteries and veins in the rats by microsurgical techniques. Chlorhexidine at 0.05%, 0.02% and 0.01% was found by contrast to have a very low toxicity which was comparable to physiological saline[76].
26. 2 ml. of 0.05% chlorhexidine was injected intraperitoneally in rats, 5 minutes after the inoculation of pure Escherichia coli. The percentage of neutrophils was superior to the control group[78].

Human

27. Absorption after oral ingestion is very low and long term oral use has not produced changes in haematological and biochemical parameters[64].
28. People who are bathing every day of their lives for years with chlorhexidine have no ill effects[61].
29. While 0.5% chlohexidine is irritant to the bladder of some people, 0.1% is not[61].

Povidone Iodine

It is an effective broad-spectrum disinfectant with no reported toxicity to cornea and conjunctiva when applied topically in single dose to ocular surface[18]. Gram negative organisms are particularly susceptible[20]. Povidone iodine is effective against *Neisseria gonorrhoea, Chlamydia trachomatis*, Herpes simplex virus type-ll with three different concentrations (5%, 1% and 0.1%)[18]. It is Germicidal in action. It has a broad-spectrum bactericidal activity and is also effective against fungi. As the incidence of fungal infection of the cornea increases with the use of antibiotics and is more dangerous in presence of steroids, if these drugs are to be used for prolonged periods, topical administration of 1% povidone-Iodine reduces the danger of superimposed mycotic infection[17]. It significantly reduces the duration of contamination by the fungus. Povidone-Iodine 5% solution is effective in reducing bacterial recovery from the perilimbal conjunctiva, where most incisions for intraocular surgery occur[19].

Polyhexamethylene biguanide (PHMB)

It is used as an environmental disinfectant under trade names such as cosmocil CQ, Vantocil IB, and Baquacil, varying in purity. Baquacil is available as a swimming pool disinfectant in the USA; it is an effective sanitiser and is algistatic and amoebicidal[39]. Cosmocil CQ is used for the preservation of cosmetics and pharmaceuticals, and PHMB is in some soft-contact-lens disinfectant solutions. PHMB is not manufactured or licensed for therapeutic use. It is generally less effective than chlorhexidine in vitro against Acanthamoeba cysts.[39]

Silver sulphadiazine (SSZ)

It is a substitute for silver nitrate and does not cause argyrial stain. It has a broad spectrum coverage against bacteria, fungus & viruses[33]. SSZ 1% eye ointment 5 times

a day for 2-3 weeks has been recommended. Silver sulphadiazine combines the oligodynamic action of silver with the antibacterial effect of sulphadiazine. It is observed that sulphonamide antagonist para aminobenzoic acid (PABA) did not nullify silver sulphadiazine action and that the silver moiety combined in vitro with both DNA and cell membrane[33]. Advantages were thought to be the absence of side effects, economy and it's efficacy in deeper and extensive lesions[29]. It inhibits the replication of pathogens. 1% silver sulphadiazine eye drops are also available[33]. In practice, this preparation is no longer being used in India.

3.5. Antiacanthamoebal drugs

1.Diamidines:-

Propamidine
Dibromopropamidine
Hexamidine
Iodohexamidine

Propamidine (Brolene)

Mr. Peter Wright and Dr. David Warhurst recorded the first medical cure of acanthamoebal keratitis by employing only the topical administration of dibromopropamidine ointment (0.15%), propamidine isethionate eye drops (0.1%), and neomycin eye drops. The minimal inhibitory concentration (MIC) and the minimal amoebicidal concentration (MAC) for propamidine were each less than 1.25 μg per millilitre, and for dibromopropamidine the concentrations were less than 1.25 μg per millilitre (MIC) and 2.5 μg per millilitre (MAC), respectively[4]. They are analoges of stilbamidine and had previously known antibacterial and antifungal effects[4].

Chlorhexidine

In high concentrations it effectively kills cysts of Acanthamoeba sp[36]. 0.02% chlorhexidine digluconate drops in 0.9% physiological saline in combination with 0.1% propamidine isethionate (Brolene) drops, given hourly by day and night for the first 3 days, then 2 hourly by day for 4 weeks, 3 hourly by day for 4 weeks and 4 hourly by day for 4 months had already been recommended for the treatment of acanthamoeba keratitis[39].

Although compounds such as hydroxystilbamidine, paromomycin, neomycin and miconazole have been found to have variable activity in vitro there has been no published report of medical cure[4]. The disease may be arrested with the use of orally administered ketoconazole and flucytosine, and topical clotrimazole ointment. The disease was arrested after penetrating keratoplasty, orally administered ketoconazole, miconazole eye drops and cryotherapy.[4] Acanthamoeba is said to be sensitive to natamycin that disrupts the cell membrane by binding with ergosterol[53]. Polymyxin B produces a disorientation of lipoproteins in the cell membrane, permitting excess permeability[53]. Acridine dye is believed to bind to mitochondrial DNA where it inhibits the synthesis of proteins essential for cellular respiration[53].

3.6. Fungal Culture and sensitivity testing.

Ideally all antimicrobial agents should be discontinued 24 to 48 hours before taking corneal scrapings[31]. The Kimura platinum spatula is ideal but a 21 gauge needle may be used for corneal scrapings. Preservative free proparacaine hydrochloride (0.5%) is less antiseptic than other topical anaesthetics. It is best to use more than one culture media, preferably both solid and liquid, and incubate these media at 37° C and 25°C to 30° C. According to Liesegang and Forster fungal growth usually occurs within 48 to 72 hours after inoculation but O'Day et al recommend that the culture media should be kept for 6 to 8 weeks[54]. Solid media are inoculated by lightly streaking the spatula or loop over the agar surface in rows of C-streaks. Growth on the C-streaks is considered to be significant, growth away from C-streak is considered to be contaminated. Liquid media are inoculated by twirling the loop or spatula in the broth several times[54]. Sabouraud's dextrose agar with gentamicin (50 microgram/ml) is the primary isolation medium for fungi. The medium is nutritionally deficient but the low pH favours fungal growth.[31] Chloramphenicol or penicillin plus streptomycin may be incorporated in to the Sabouraud's dextrose agar medium to suppress bacterial growth[54].

Sabouraud's dextrose agar media (SDA).

Preparation: 65 gm. suspended in 1 litre of distilled water and boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minute. Antibiotics are used to suppress bacterial growth[22].

Used with drugs:

Before autoclaving: 0.4 gram. chloramphenicol, 0.5 Gram cycloheximide are added to each litre of reconstituted medium.

After autoclaving: 0.5 gram cycloheximide, 20,000 unit penicillin and 40,000 unit streptomycin are added to each litre of autoclaved cooled medium. Chloramphenicol is used to minimise bacterial contamination and cycloheximide to reduce contamination with saprophyte fungi. Cycloheximide can not be used in all media as some pathogens for example *Aspergillus, Histoplasma, Hendersonula, Cryptococcus, Candida* are inhibited by it[22].

Brain-heart infusion broth with gentamicin enhances the isolation of filamentous fungi and yeast[31]. Blood and chocolate agar media support the growth of the majority of fungi at 35°C to 37°C under increased carbon dioxide[31]. Thioglycollate or thiol broth is a semisolid medium that provides adequate redox potentials for the growth of aerobic fungi as well as aerotolerant anaerobes, microaerophillic bacteria, facultative anaerobes and aerobic bacteria[31].

To do antifungal sensitivity tests first prepare the drug solutions, media and inoculum.

1. Preparation of stock drug solution

To prepare a drug stock solution (1280 mg/litre), 64 mg of the drug is dissolved in 50 ml of distilled water[42]. This solution should be filter sterilised and can be stored in small amounts at -20°C for up to 12 months.

2. Preparation of media.

Broth or agar, according to fungus to be tested e.g. yeast or filamentous fungi. Can be obtained from Difco or Oxoid company.

3. Control isolates

Can be obtained from the Mycological Reference Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9, UK[42].

4. Preparation of inoculum

The yeast inoculum should be prepared from overnight (18 hours) to 48 hours old cultures on Yeast Morphology Agar. 2 ml amounts of sterile distilled water are inoculated of with a loopful of each isolate and the concentration of the suspension adjusted to about 1 x 10^6 cells/ml[42]. When filamentous fungi are being tested, the inoculum should be obtained from 2 to 5 day cultures on Sabouraud's glucose agar. Fungal suspension is prepared by immersing the surface growth in sterile distilled water containing 0.05% Tween 80 and scraping off spores and hyphae with a sterile bent glass rod or wire loop. A spectrophotometer is set at 530 nm to adjust the suspension to 90% transmittance (T). This should result in a concentration of about 1 x 10^6 colony forming units (CFU)/ml. To achieve this density of fungus, it may be necessary to concentrate the original suspension by centrifugation[42].

1. Disc diffusion method (DD)

This is the simplest method in which the drug is allowed to diffuse from an impregnated paper disc placed on an agar medium which has been inoculated with the strain under investigation. 15 ml of molten agar is placed in 9 cm diameter petridishes. Once the agar is solidified and dry, the plates are inverted and a line on the base is drawn so as to bisect it. With a sterile swab one half is inoculated with the suspension ($1x10^6$ cells/ml) of sensitive strain and other half with a test isolate. Then the disc is placed in the centre of each plate and the plate incubated. After 48 hours of incubation the diameter of zone of inhibition is measured both in test isolate and the sensitive strain. Then the percentage difference is calculated as follows[42]:

$$\frac{\text{test isolate zone diameter}}{\text{control strain zone diameter}} \times 100$$

Interpretation of results

Isolates with zones of inhibition which are not less than 80% of the control strain diameter can be regarded as sensitive. Resistant isolates will often grow up to the disc edge, or almost so. Isolates which give zones that are less than 80% of the control strain diameter are often susceptible to treatment[42]. Intermediate zones also suggest the increased likelihood of the emergence of resistance during treatment. The presence of discrete colonies within otherwise clear zones of inhibition is another pointer to the development of resistance.

2. Broth dilution method

In broth dilution tests serial dilutions of the drug are prepared in a fluid medium and then inoculated with a suspension of the fungus under investigation. The drug stock solution (1280 mg/litre) is ten-fold diluted by adding 1 ml to 9 ml of broth[42]. The final concentration is 128 mg/ litre. 11 sterile capped tubes (110 x 16 mm) are placed in a rack and numbered 1 to 11. 1 ml of broth is added to each tube. Then 1 ml of 128 mg/ litre drug solution is added to tube-1 and mixed. Then 1 ml of solution from tube-1 is transferred to tube-2. This serial dilution is repeated through tube-9 and 1 ml from tube-9 is discarded. Then 50 microlitre of the standardised inoculum is added to tube 1

to tube-9 and incubated. Tube-10 is the medium control and tube-11 is the inoculum control. Drug dilution in tube-1 is 64 mg/litre and in tube-9 it is 0.25 mg/ litre.
The MIC is the lowest drug concentration at which there is no visible fungal growth after 48 hours incubation. Growth must be present in the medium control and absent in the inoculum control[42].

3. Agar dilution method

2 ml of concentrated agar solution is added to each of 10 sterile universal container and numbered 1 to 10. 2 ml of the 1280 mg/litre stock drug stock solution is added to container-1, mixed well and transferred 2 ml to container-2 and repeated through container-9. From container-9, two ml is discarded. Melted 200 ml agar is dispensed 18 ml each in 10 sterile universal container placed in 56°C water bath and numbered 1 to 10. Now 2 ml of the corresponding drug containing solutions added to each container of molten, cooled agar, mixed well and poured the contents in to a 9 cm diameter petridishes and numbered 1 to 10. Once the medium is solidified and dried, with a micropipette 20 microlitre of standardised suspension is inoculated and incubated for 48 hours. Plate-10 is medium control and drug dilution in plate-1 is 64 mg/litre and in plate-9 it is 0.25 mg/litre.
MIC is the lowest drug concentration at which there is no visible fungal growth after 48 hours incubation. Growth must be present in the medium control[42].

4. Bioassay

Drug standards are prepared according to the standard method. Media are prepared according to the organisms to be tested. The inoculum is prepared by harvesting the spores from the culture in sterile distilled water and adjusting the concentration to about 1 x 10^6 spores/ml[42]. 1 ml of the spore suspension is added to the melted media and poured into a 25 cm sq. plate and dried. 30 wells of 4 mm diameter are cut in the media and 15 microlitre of standard specimen or patient serum is placed in each well. Each standard and specimen should be tested in triplicate and should be placed on the plate according to the randomised distribution. The plate is incubated for 24 hours. Then the zones of inhibition around each well is measured. The results are analysed by a microcomputer program.

Most antifungals do not diffuse well and give poor or misleading results even when sensitive strains are tested against high drug concentrations. The lowest concentration of the drug to inhibit growth after incubation is referred to as the minimum inhibitory

concentration. The minimum fungicidal concentration is defined as the lowest concentration of drug from which sub-cultures were negative. The decision whether an infection with a given fungal strain should be treated with a particular drug, or whether that strain should be regarded as resistant to that drug, is often difficult. It is not unusual to obtain MICs for responsive strains that are much higher than the levels of drug that can be attained in the patient. The MIC obtained often depends on the conditions of the test, with the concentration of the fungal inoculum, the composition and pH of the medium, and the temperature and length of incubation all having a marked effect on the result[42]. The results of MIC determinations must be interpreted with caution because their correlation with clinical effectiveness is often uncertain[42].

4.0. METHODS

4.1. In vitro sensitivity tests:

In the developing countries fungal keratitis is very common. On the other hand there are usually no microbiological facilities to identify the micro-organisms or to do the susceptibility tests. The aim was to develop a very simple method for sensitivity tests which could also be used in a small hospital in a developing country. Cheap antiseptic agents such as chlorhexidine, propamidine, povidone iodine and polyhexamethylene biguanide (PHMB), were compared with econazole and natamycin and some antibacterial agents like gentamicin, chloramphenicol and ciprofloxacin.

The sensitivity tests can be done in normal agar media in petridishes or in any media. Sabouraud's dextrose agar (SDA) media is preferred to do susceptibility tests for filamentous fungi. Four wells are made in the media in petridishes by a metallic borer and are marked accordingly. The size and shape of all the wells are same, 7 mm in diameter and 2.5 mm in depth which depends on the thickness of the media in the petridishes. The diameter of the petridish is 9 cm. Then with a swab stick the microorganisms are wiped all over the media containing wells carefully, so that the wells are not disturbed or broken. After the inoculation is done, with disposable pipettes wells are filled with the antimicrobial agents as per marked earlier. The wells are filled in such a way that the fluid should be in the same plane as the media. It should not over flow the surface of the media. Then the cultures are incubated at 28°C for 24 to 48 hours and in case of bacteria they are incubated at 37°C for 24 hours. After incubation, diameters of the zones of inhibition around the wells are measured in mm by a scale and 7 mm was subtracted. The method is very simple and could be done in any isolated situations where microbiological facilities are not available and we call it the well diffusion (WD) method. The method of sensitivity is shown in the Illustration-1

Specimens sent from Aravind Eye Hospital, India, and Korle Bu Teaching Hospital, Accra, Ghana, were cultured on Sabouraud's dextrose agar media in the microbiology laboratory of the Department of Pathology, Institute of Ophthalmology, London. The sensitivity tests for the fungi were done against chlorhexidine 0.2%, 0.1%, 0.05%, 0.02%, propamidine (Brolene) 0.1%, chlorhexidine 0.2% plus propamidine 0.05%,

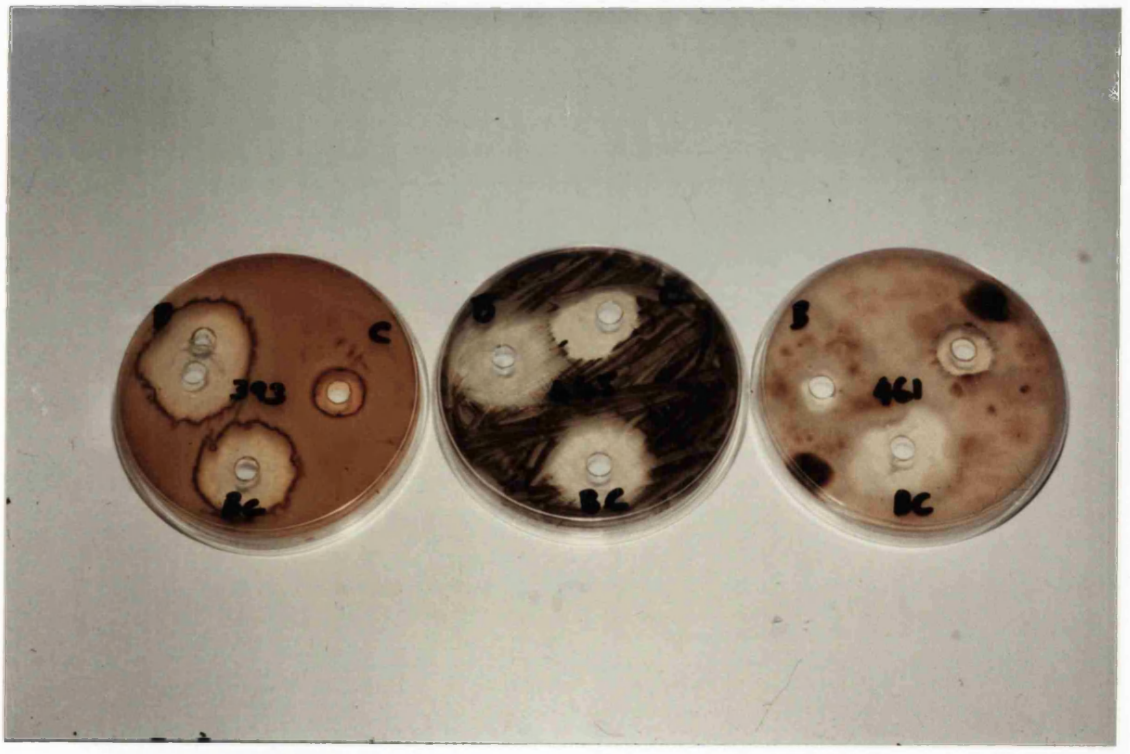
PHMB 0.02%, povidone iodine 1%, 2%, 5%, chlorhexidine 0.2% plus povidone iodine 2.5%, and econazole 1%.

Forty specimens of fungi from the randomised trial of different concentrations of chlorhexidine and natamycin 5% were tested against 0.05%, 0.1%, 0.2% chlorhexidine and 5% natamycin in the Aravind Eye Hospital, Madurai, India. Corneal scrapings were cultured in dishes containing potato dextrose agar media (PDA) and blood agar media (BA). If there were no growth within 2 weeks of time the plate was discarded. Antibacterial sensitivity tests were also done using 13 different species of bacteria from the stock culture available in the Aravind Eye Hospital, against gentamicin, ciprofloxacin, chloramphenicol and chlorhexidine 0.05%, 0.1% and 0.2%. Gentamicin, ciprofloxacin and chloramphenicol were purchased from the local pharmacy and chlorhexidine 0.05%, 0.1% and 0.2% solutions were prepared from 20% chlorhexidine solution. *Streptococci* and *Pneumococci* were taken from the stock culture in to the brain heart infusion broth and the other bacteria were taken in to the peptone water containing peptone 10 gm and sodium chloride 5 gm in 1 litre of water. *Streptococci* and *Pneumococci* were sub-cultured in blood agar media and other bacteria were sub-cultured in the Mueller Hinton agar media. The cultures were incubated at 37°C for 24 hours. Sensitivity tests of gentamicin, chloramphenicol and ciprofloxacin were done by both disc diffusion (DD) and well diffusion (WD) methods.

In the randomised trial of chlorhexidine and natamycin 2.5%, done in the EITC, Chittagong, Bangladesh, 61 fungal specimens were tested against chlorhexidine 0.2%, natamycin 2.5% and econazole 1% by the above mentioned method.

Illustration - 1

Showing the method of sensitivity testing in Sabouraud's dextrose agar plate



4.2. Pilot study of treatment

As described in the results the in vitro susceptibility tests showed that both Brolene and PHMB had disappointingly poor activity against fungi. It is unlikely that these agents will have a role in the treatment of mycotic keratitis. As expected econazole proved very effective. Chlorhexidine exhibited a dose related response. Povidone iodine appeared useful at all concentrations and mixing it with chlorhexidine gives activity of some degree against all isolates although with a reduced average zone size compared with the results of even 1% povidone iodine alone. So 0.2% chlorhexidine, 5% povidone iodine and 1% econazole were chosen for a clinical pilot study.

Consecutive patients with suppurative corneal ulcers attending the cornea clinic of Aravind Eye Hospital, Madurai, India were examined clinically and corneal scrapings were taken by Kimura spatula for Gram staining, 10% potassium hydroxide test and culture. The patient was selected for study if there was microscopic observation of hyphal fragments either in the 10% potassium hydroxide mount or in Gram stained smear. The fungi were cultured in potato dextrose agar media (PDA) and were incubated at room temperature (25°C-28°C) for two weeks. After that if there was no growth we considered it as negative. After taking informed consent from the patient or from his or her guardian that the patient was willing to be included in the study, would stay in the hospital for 7 to 10 days and would come back for follow up after 2 weeks, only then was the patient admitted to the hospital for study. The drops were given every hour for 5 to 7 days or until the ulcer was healing or signs of deterioration was evident in the hospital and every 3 hour after discharge for one week until follow up.

The ulcer was categorised as nonsevere when the ulcer had a diameter of less than 6 mm with ulceration of the superficial one-third and suppuration of the superficial two-thirds of the cornea, without either chance of perforation or scleral suppuration. It was categorised as severe when the ulcer had a diameter of 6 mm or more with ulceration and suppuration involving the deep one-third of cornea with the possibility of perforation and scleral suppuration.[37,38] If posterior corneal abscess or endothelial plaque were present the ulcer was also categorised as severe.

Signs of improvement were as follows: (a) Blunting of the margins of ulcers. (b) Improvement of signs of inflammations. (c) Reduction in cellular infiltrate and oedema. (d) Reduction in corneal epithelial defect. (e) Signs of re-epithelialisation. (f) Reduction in anterior chamber hypopyon if present. (g) Decreased complaint of pain

by the patient. Atropine or homatropine drop was instilled 2-3 times a day regularly. Some times oral analgesics were given if necessary. The drops were given from 7 am to 10 pm. 0.2% chlorhexidine, 5% povidone iodine and 1% econazole were supplied by the pharmacy of the Moorfields Eye Hospital, London in sealed bottles. The drugs were stored at 4°C to 10°C in the microbiology laboratory, Aravind Eye Hospital, India. The patient was examined daily and findings were recorded on the 2nd, 5th, 7th and sometimes 10th day if still in hospital, and at follow up on the 14th day. If there was no improvement or clear cut signs of deterioration of ulcer within 5 days or there were signs of toxicity of the medication, we changed the drug from chlorhexidine and povidone iodine to econazole. If the chosen drug failed to improve the ulcer the patient was treated according to standard hospital procedure (5% natamycin eye drops).

The effect of the drug was expressed as (a) no response (b) healed or (c) perforated. No response means all symptoms and signs the same as when recorded at presentation or getting worse, i.e.: increasing ulcer size, infiltration or hypopyon. Healed means there is scar formation, with no epithelial defect with fluorescein stain, no infiltrate, no hypopyon and improvement in vision or vision no worse than baseline level.

Signs of toxicity were as follows: (a) Patient's intolerance such as pain, burning sensation etc. (b) Swelling of the eye lids. (c) Increased conjunctival congestion and chemosis. (d) Conjunctival staining with fluorescein. (e) Punctate corneal epithelial erosion.

4.3. Randomised trial of different concentrations of chlorhexidine and natamycin 5%.

The in vitro studies in London showed that almost all the isolates of fungi were sensitive to povidone iodine, chlorhexidine and econazole. The response increased with the increased concentrations of chlorhexidine. However, in the pilot study of treatment, in cases which were all positive for fungus on smear and culture in the Aravind Eye Hospital, Madurai, India, chlorhexidine 0.2% performed better than 1% econazole and 5% povidone iodine. Out of eleven patients treated with chlorhexidine 0.2%, ten were healed. No adverse symptoms to any of these drugs were experienced and there was no toxicity. Therefore a randomised trial was started to determine the safety and efficacy of different concentrations of chlorhexidine in the treatment of fungal corneal ulcers.

We studied a total of 60 patients. Patients attending in the cornea clinic of Aravind Eye Hospital, Madurai, India, with suppurative corneal ulcers were scraped by Kimura spatula for 10% potassium hydroxide test, Gram stain and culture. Microscopic observation of fungal hyphal fragments in 10% potassium hydroxide mount or in Gram stain made the patient eligible for the trial. Patients with only one eye, diabetes mellitus, already perforated eye, mixed bacterial and fungal infection and unwilling to participate in the study were excluded. After giving informed consent the patient was recruited for study.

There were four arms of the study, three concentrations of chlorhexidine namely 0.05%, 0.1% and 0.2% and natamycin 5%. The randomisation was computer generated using the one sample run test. The bottles were labelled only with the randomised numbers. 20% solution of chlorhexidine was supplied by the Moorfields Eye Hospital, London and diluted with water in Madurai, natamycin 5% was purchased from the local pharmacy. Preparation and labelling of drugs were done by the Aravind eye hospital authority. The prepared bottles were preserved in 4°C to 8°C. The eligible patients were treated in the first day 1 drop half hourly for 3 hours then hourly for the rest of the day during waking hours. From the second day onwards drops were given 2 hourly during waking hours for 5 days. After that 3 hourly drops were used for a further two weeks giving a total 3 weeks of medication. A log book was maintained for dispensing drugs to the patients. If there was no response by 5 days, clear cut signs of deterioration of ulcers or any untoward reaction to the medication developed then the drug was withdrawn and management continued as per

the standard hospital procedure (natamycin 5%). Each patient was given a discharge summary with the date on which he should come back to the hospital for review. A post card was mailed 5 to 7 days prior to the due date as a reminder to the patients. Even after this if they fail to come an Ophthalmologist went with a social worker for follow up in the field. If, in spite of all these efforts, the patient failed he was considered as a "lost to follow up" case. For this follow up transportation costs either by bus or by train were given to patients and some money to buy food to some poor patients during their stay in hospital. Data were recorded on the 2nd, 5th, 7th day of study and any time between 2nd and 3rd week for follow up.

The statistical analysis was done on the basis of the severity of ulcers, the baseline characteristics of patients, such as age, sex, literacy, home, prior treatment, trauma and duration of ulcers. The ulcer was categorised as nonsevere when the ulcer had a diameter of less than 6 mm with ulceration of the superficial one-third and suppuration of the superficial two-thirds of the cornea, without either chance of perforation or scleral suppuration. It was categorised as severe when the ulcer had a diameter of 6 mm or more with ulceration and suppuration involving the deep one-third of cornea with the possibility of perforation and scleral suppuration. If posterior corneal abscess or endothelial plaque were present the ulcer was also categorised as severe. The results were analysed by outcome of treatment at day 5, unstratified, stratified by severity of ulcers and stratified by patients having had prior antifungals or not. The data were also analysed by outcome of treatment at day 21, excluding the severe ulcers.

The ratio of the 4 proportions of succesful outcomes were expressed as relative efficacy (RE), simply dividing the results with different concentrations of chlorhexidine by results with natamycin (chlorhexidine / natamycin), with natamycin as referent. 95% confidence intervals were calculated for relative efficacy. P-value was calculated.

This was done through analysis of data stratified by levels of severity and by prior antifungal treatment, using the Mantel-Haenszel method. Adjusted estimates of the efficacy ratios with 95% confidence limits and p-values are reported.

4.4. Randomised trial of 0.2% chlorhexidine and 2.5% natamycin

From the successful clinical trial of different concentrations of chlorhexidine in the Aravind Eye Hospital, Madurai, India, it was seen that the best results were obtained with 0.2% chlorhexidine without any toxicity. As chlorhexidine is inexpensive it may be the best first line drug for suppurative keratitis in the tropical developing countries. So we decide to conduct a clinical trial in the Eye Infirmary and Training Complex (EITC), Chittagong, Bangladesh, with 0.2% chlorhexidine as compared with 2.5% natamycin which is the standard treatment of fungal keratitis there.

Patients attending the cornea clinic of EITC, Chittagong, Bangladesh with suppurative keratitis were scraped for 10% potassium hydroxide test, Gram stain and culture. Microscopic observation of fungal hyphal fragments in 10% potassium hydroxide mount or in Gram stain were necessary for recruiting the patients. Patients with only one eye, diabetes mellitus, already perforated eye, unwilling to participate in the study, children under 1 year age and mixed infections were excluded. There were two arms of the study, 0.2% chlorhexidine and 2.5% natamycin. The randomisation was computer generated in London and the codes A for natamycin and B for chlorhexidine were sealed in the envelopes. After recruitment of patients the envelopes were opened serially. The envelopes were numbered serially from 1 to 100. Passport-type Polaroid photographs of each patient was taken for future identification and were attached on the respective records. 20% chlorhexidine solution was supplied by the Moorfields Eye Hospital, London and diluted with water in Chittagong and 2.5% natamycin was available in the EITC as their standard treatment for fungal keratitis. Preparation and labelling of chlorhexidine was done by the EITC authority and the bottles were kept in 4°C. Corneal scrapings were cultured in the SDA, chocolate agar media (CHA), and blood agar media. If there were no growth on the 14th day the cultures were discarded. The sensitivity tests were done by the well diffusion method using 0.2% chlorhexidine, 2.5% natamycin and 1% econazole.

After taking informed consent the eligible patients were treated in the first day 1 drop half hourly for three hours, then 1 hourly for 2 days, 2 hourly for 5 days and 3 hourly for 2 weeks, a total 3 weeks treatment. The drops were given only in the waking hours. A log book was maintained for dispensing medicines to the patients. If there was no response by 5 days, clear cut signs of deterioration of ulcers or development of any untoward reactions the drugs were withdrawn and were managed as per standard hospital procedure. 5% natamycin drop, 1% econazole drop and 1% clotrimazole ointment were used as back-up. The eyes were examined daily and the findings were

recorded on 2nd, 5th, 7th day and anytime during the next 2 weeks. A close-up camera was used to take the photographs of the corneal ulcers on the 1st, 5th, 7th day and any time during the next 2 weeks. Each patient was given a discharge summary with the date on which he should come back to the hospital for review. A post card was mailed 5 to 7 days prior to the due date as a reminder to the patients. Even after this if they failed to come an Ophthalmologist went to see some patients in their homes and the rest were brought to the hospital for follow-up. In spite of all these efforts a few failed to be seen and were considered as a lost to follow-up case. For this follow-up travel costs were given to the patients.

The statistical analysis was done on the basis of the baseline characteristics of the two treatment groups such as age, sex, prior antibiotic, prior treatment, ulcer duration, ulcer size, epithelial defect (A. less than 2 mm, B. 2-4 mm, C. 4-6 mm, D. 6-8 mm), ulcer depth (A. superficial 1/3, B. superficial 2/3, C. deep), hypopyon (A. no, B. 0-2 mm, C. 2-5 mm, D. more than 5 mm), infiltration (A. 0-30%, B. 30-60%, C. 60-100%), deep lesion (A. none, B. posterior corneal abscess, C. endothelial plaque), severe ulcer and perforation threat (yes or no). The ulcer was categorised as nonsevere when the ulcer had a diameter of less than 6 mm with ulceration of the superficial one-third and suppuration of the superficial two-thirds of the cornea, without either chance of perforation or scleral suppuration. It was categorised as severe when the ulcer had a diameter of 6 mm or more with ulceration and suppuration involving the deep one-third of cornea with the possibility of perforation and scleral suppuration. If posterior corneal abscess or endothelial plaque were present the ulcer was also categorised as severe. The data were analysed by outcome of treatment at day 5, stratified by ulcer size by multiplying two dimensions (1-12 sq. mm & > 12 sq. mm). The data was also analysed by outcome of treatment at day 21.

The ratio of the outcomes of the 2 proportions were expressed as relative efficacy (RE), simply dividing succesful results with chlorhexidine by outcome with natamycin (chlorhexidine / natamycin). 95% confidence limits were calculated from relative efficacy. P-value was calculated. Adjustment was made for the confounding effect of the following factors:
1. Age. 2. Sex. 3. Duration of ulcer. 4. Prior antibiotic treatment. 5. Severity of ulcer. 6. Ulcer size. 7. Degree of epithelial defect. 8. Depth of ulcer. 9. Degree of hypopyon. 10. Degree of infiltrate. This was done through analysis of data stratified by levels of the confounders, using the Mantel-Haenszel method. Adjusted estimates of the efficacy ratios with 95% confidence limits and p-values are reported.

5.0. RESULTS

5.1. In vitro sensitivity tests:

Sixty three isolates from India and thirty two from Ghana listed in Appendix 2 to 7 were tested in London. Isolates from India included *Fusarium spp.* 13 (21%), *Aspergillus spp.* 12 (19%), *Coelomycete* spp. 7 (11%), *Exserohilum* spp.4 (6%), *Acremonium* spp. 4 (6%), with 9 other species making up 13 (21%) and 10 (16%) as yet unidentified. The detailed results are presented in the Appendix-2 to 4. Isolates from Ghana included *Fusarium spp.* 18 (57%), *Aspergillus spp.* 5 (16%), with 5 other species making up 7 (21%) and 2 (6%) unidentified. The detailed results are presented in the Appendix-5 to 7. The summarised sensitivity test results are shown in the Table-2 and in Graphs 2 to 5. The list of fungi are shown in Table-3.

Table-2

Summary of results of antifungal drug sensitivity tests against fungi from Aravind Eye Hospital, India and Ghana done in UK.

Name of antifungal drugs	No. of fungi tested	No. of positive responses	No. of negative responses (No zone of inhibition)	Total zone of inhibition in mm	Mean zone of inhibition in positive cases in mm
0.02% chlorhexidine	89	39 (43.82%)	50	189	4.84
0.05% chlorhexidine	90	61 (67.77%)	29	293	4.80
0.1% chlorhexidine	94	76 (80.85%)	18	392	5.15
0.2% chlorhexidine	95	90 (94.73)	05	522	5.80
0.2% chlorhexidine + 0.05% Brolene	95	90 (94.73%)	05	710	7.88
0.1% Brolene	95	41 (43.15%)	54	447	10.90
0.02% PHMB	93	20 (21.50%)	73	144	7.20
1% econazole	93	90 (96.77%)	03	1421	15.78
1% povidone iodine	95	78 (82.10%)	17	661	8.47
2% povidone iodine	95	88 (92.63%)	07	962	10.93
5% povidone iodine	93	88 (94.62%)	05	1045	11.87
0.2% chlorhexidine + 2.5% povidone-iodine.	92	92 (100%)	00	712	7.73

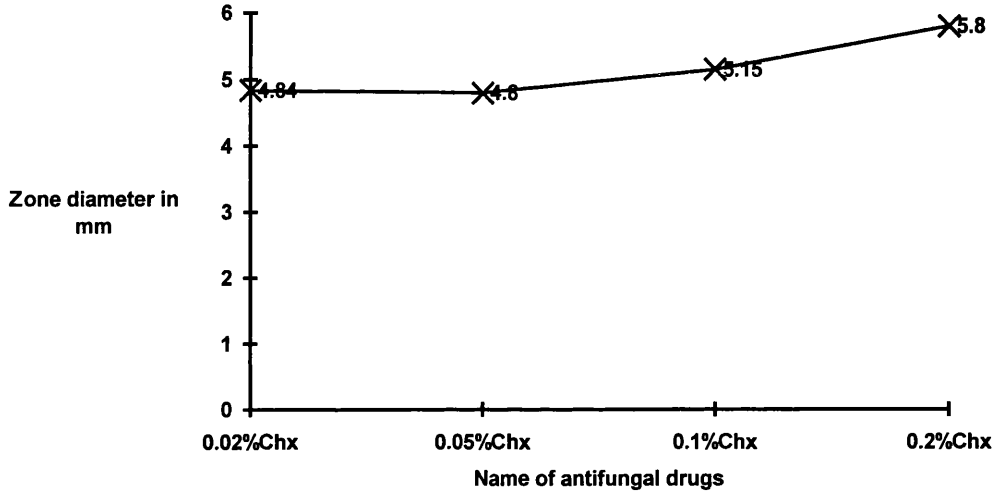
Table-3

Isolates of fungi from Aravind Eye Hospital, India and Ghana in the in vitro sensitivity tests done in UK.

Name of organisms	Total no.	Percentage
Fusarium sp.	31	32.63%
Aspergillus sp.	17	17.89%
Coelomycete sp.	07	7.36%
Exserohilum sp.	04	4.21%
Acremonium sp.	04	4.21%
L. theobrome	05	5.26%
Alternaria sp.	02	2.10%
Curvularia sp.	03	3.15%
Cylindrocarpon sp.	01	1.05%
Exophiata jeanselmei	01	1.05%
Scedosporium	01	1.05%
Gliocladium sp.	01	1.05%
Dreschslera sp.	01	1.05%
Graphium sp.	01	1.05%
Cladosporium spp.	02	2.10%
Nigrosporium spp.	01	1.05%
Phoma spp.	01	1.05%
Unidentified fungi	12	12.63%

Graph-2

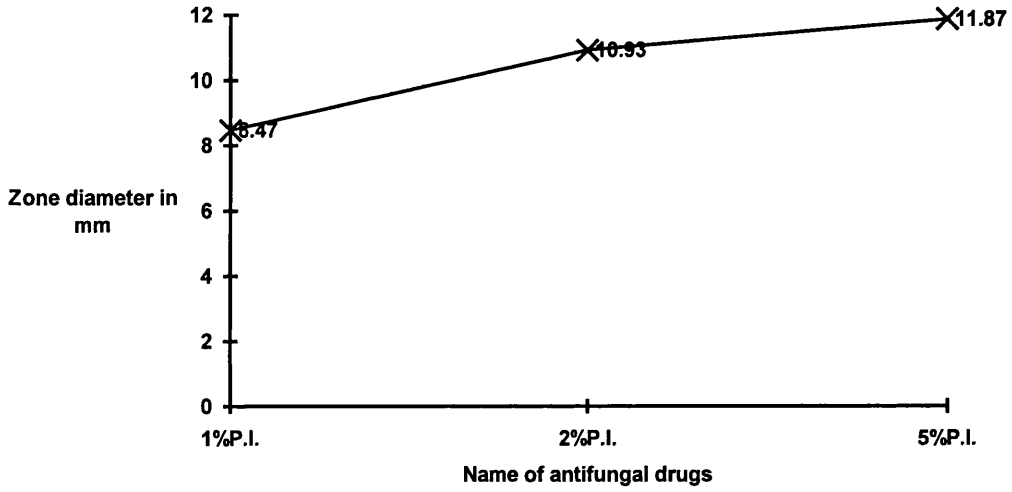
Mean zone of inhibition in positive cases of different concentrations of chlorhexidine tested against fungi from Aravind Eye Hospital, India & Ghana done in UK.



Chx = chlorhexidine

Graph-3

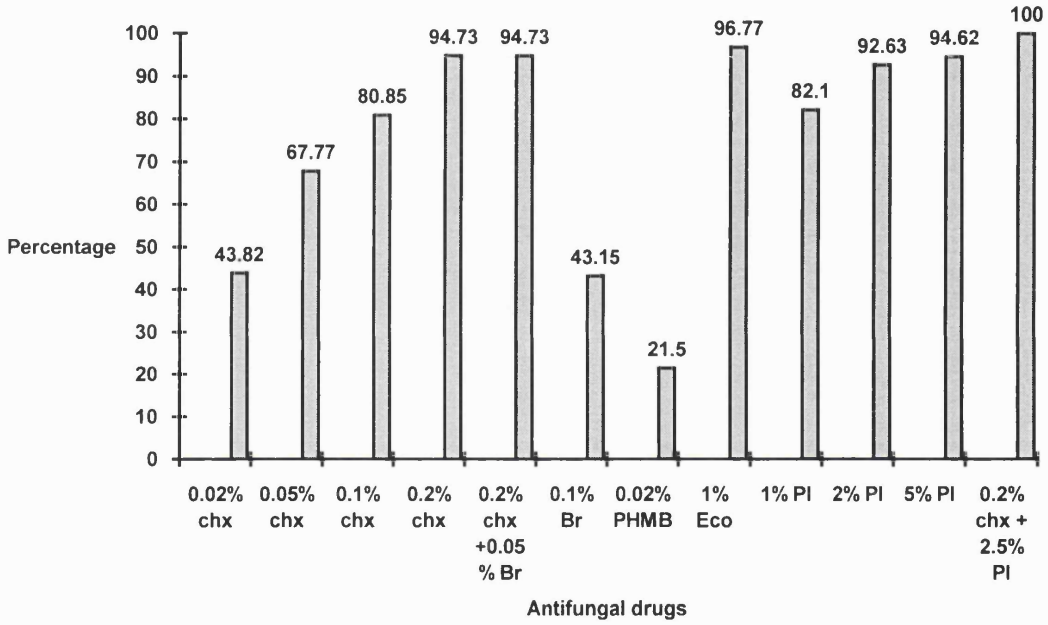
Mean zone of inhibition in positive cases of different concentrations of povidone iodine tested against fungi from Aravind Eye Hospital, India & Ghana done in UK.



P.I = povidone iodine

Graph-4

Percentage of positive responses of antifungal drugs tested against fungi from Aravind Eye Hospital, India and Ghana done in UK.



Chx = chlorhexidine

Br = Brolene

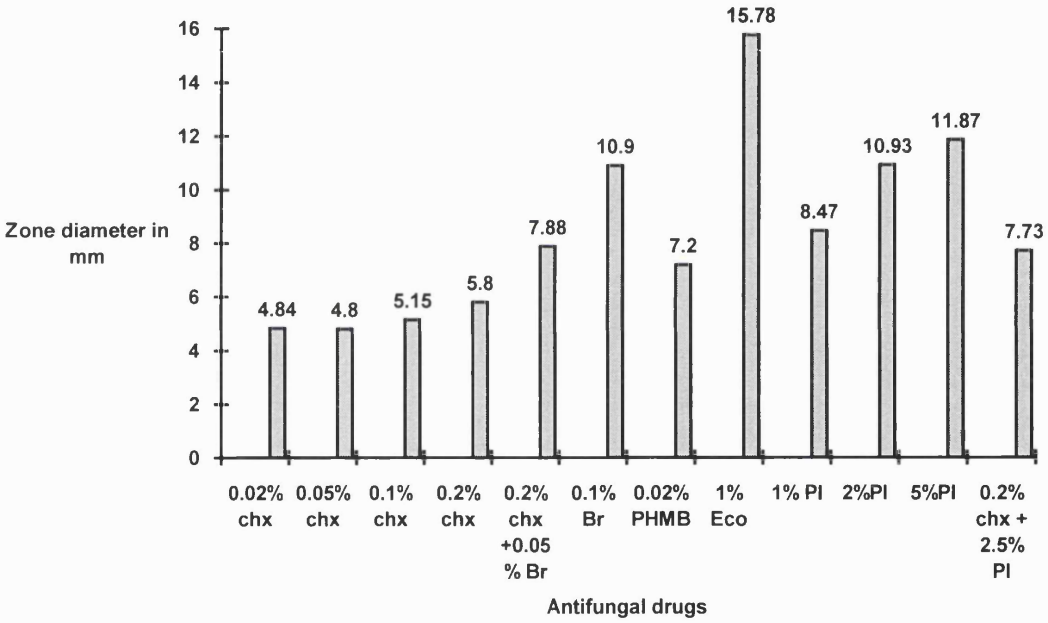
PHMB = polyhexamethylene biguanide

Eco = econazole

PI = povidone iodine

Graph-5

Mean zone of inhibition in positive cases of antifungal drugs tested against fungi from Aravind Eye Hospital, India & Ghana done in UK.



Chx = chlorhexidine

Br = Brolene

PHMB = polyhexamethylene biguanide

Eco = econazole

PI = povidone iodine

In the pilot study of treatment fungal sensitivity testing was not done. The 19 fungal isolates included *Fusarium sp.*8 (42.10%), *Aspergillus sp* 2 (10.52%), *Botryodiplodia sp.* 2 (10.52%), *Curvularia sp.* 1 (5.26%), *Bipolaris sp.* 1 (5.26%), unidentified 5 (26.31%) and there was no growth in 5 patients. The results are shown in the Table-4.

Table-4

Fungal isolates in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

			Drugs used		
Name of fungi	Total no. of fungi	Percentage	0.2% chlorhexidine	5% povidone iodine	1% econazole
Fusarium sp.	08	42.10%	04	02	02
Aspergillus sp.	02	10.51%	01	00	01
Botryodiplodia sp.	02	10.51%	02	00	00
Curvularia sp.	01	5.26%	00	01	00
Bipolaris sp.	01	5.26%	01	00	00
Unidentified fungi	05	26.31%	01	02	02

In the randomised trial of different concentrations of chlorhexidine and natamycin 5%, out of 60 patients there was no growth in 13 patients, 7 isolates were lost before the sensitivity test could be done and 40 fungal isolates were tested in India and are shown in the appendix-8. Mean zone of inhibition was 14.17 mm in natamycin 5% but there was no response in 5 cases whereas in 0.2% chlorhexidine there were no negative responses though the mean zone of inhibition was less than natamycin 5%. The summarised result is shown in Table-5 and Graphs-6&7. The fungal isolates included *Fusarium sp.* 24 (51.06%), *Aspergillus sp.* 11 (23.40%), *Curvularia sp.* 3 (6.38%), other species making 4 (8.51%) and unidentified 5 (10.63%) and are shown in Table-6.

Thirteen bacteria from the stock culture of microbiology laboratory of Aravind Eye Hospital were tested in India and are shown in Appendix-9 to 12. The summarised results are shown in Table-7 and Graph-8.

Table-5

Summary of results of antifungal sensitivity tests in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Name of drugs	Total no. of fungi tested	No. of positive responses	No response (no zone of inhibition)	Total zone of inhibition in mm	Mean zone of inhibition in positive cases in mm
0.05% chlorhexidine	40	20 (50%)	20	131	6.55
0.1% chlorhexidine	40	28 (70%)	12	207	7.39
0.2% chlorhexidine	40	40 (100%)	00	365	9.12
5% natamycin	40	35 (87.50%)	05	496	14.17

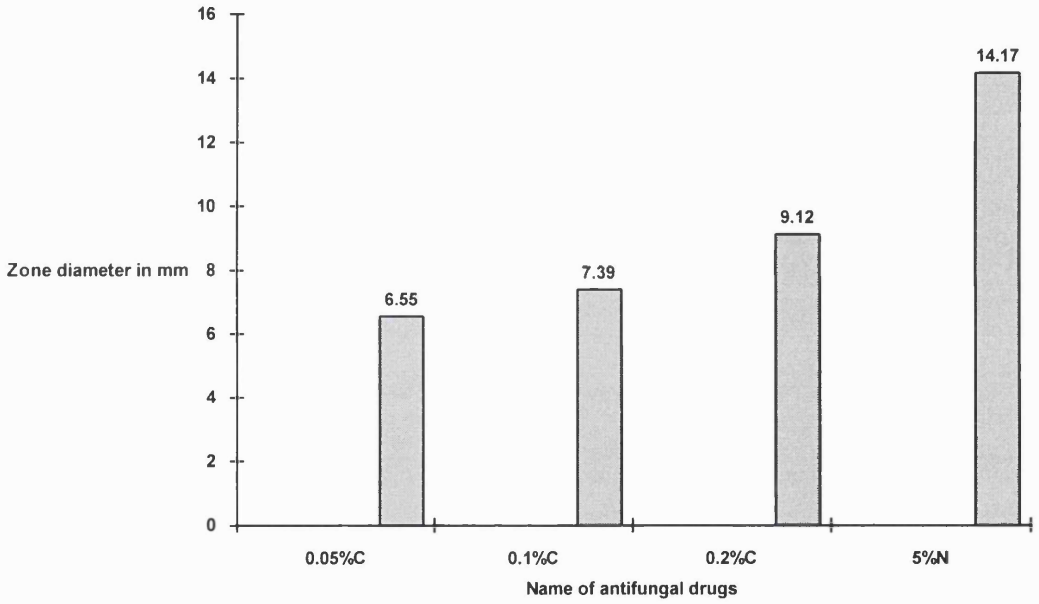
Table-6

Corneal isolates in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

			Drugs used			
Name of micro-organisms	Total no. of fungi	Percentage	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin
Fusarium sp.	24	51.06%	06	08	03	07
Aspergillus sp.	11	23.40%	04	02	00	05
Curvularia sp.	03	6.38%	01	00	01	01
Botryodiplodia sp.	01	2.12%	00	00	01	00
Rhizopus sp.	01	2.12%	00	00	00	01
Monosporium sp.	01	2.12%	01	00	00	00
Exserophilum sp.	01	2.12%	01	00	00	00
Unidentified fungi	05	10.63%	02	02	00	01

Graph-6

Mean zone of inhibition in positive responses of antifungal sensitivity tests in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

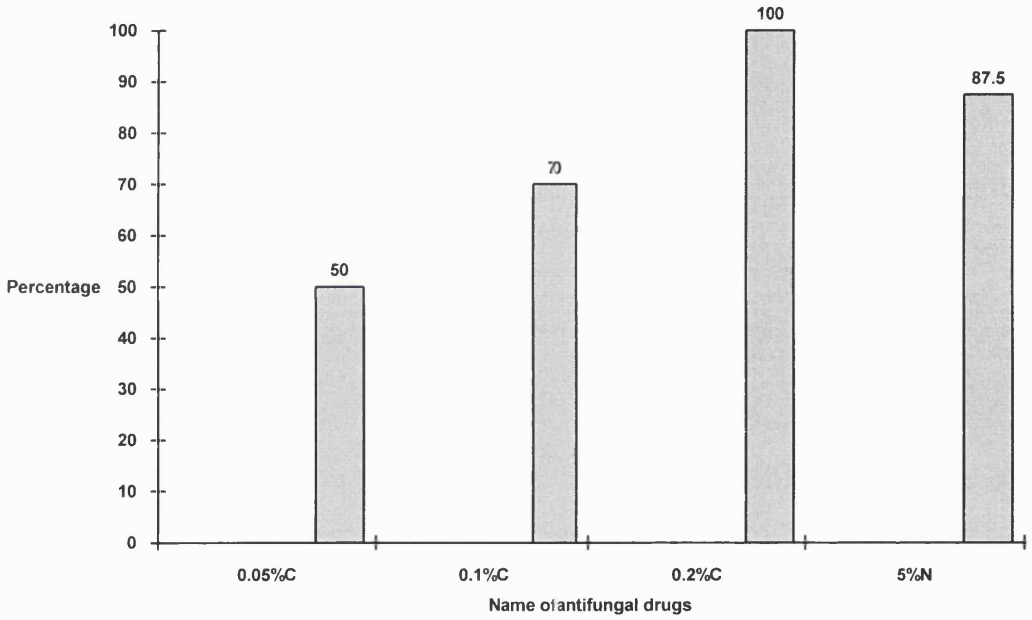


C = chlorhexidine

N = natamycin

Graph-7

Percentage of positive responses of antifungal sensitivity tests in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.



C = chlorhexidine

N = natamycin.

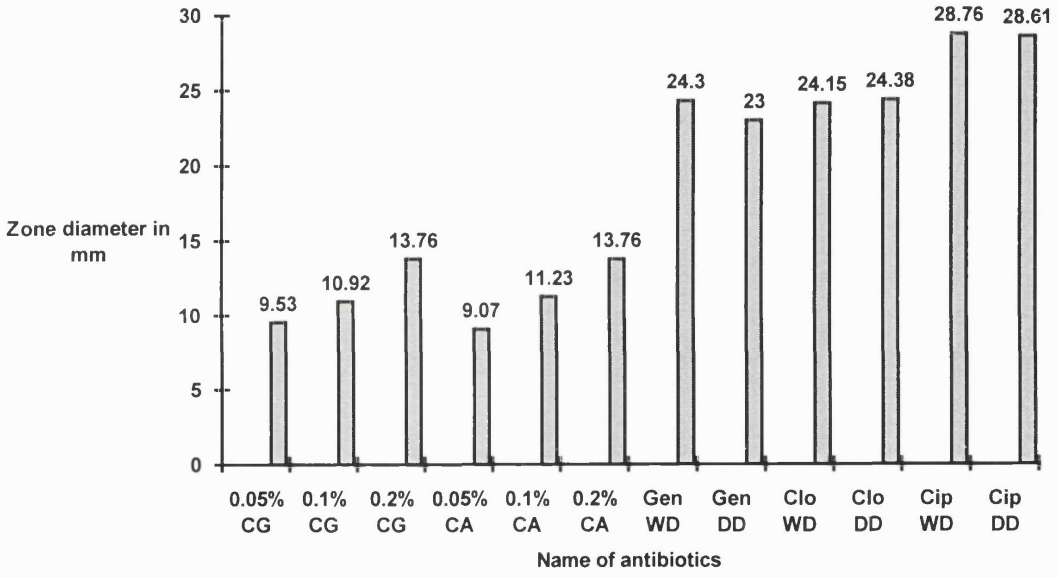
Table-7

Summary of results of antibacterial sensitivity tests done in the Aravind Eye Hospital, Madurai, India.

Name of antibiotics	Total zone of inhibition in mm	Mean zone of inhibition in mm
0.05% chlohexidine gluconate	124	9.53
0.1% chlorhexidine gluconate	142	10.92
0.2% chlorhexidine gluconate	179	13.76
0.05% chlorhexidine acetate	118	9.07
0.1% chlorhexidine acetate	146	11.23
0.2% chlorhexidine acetate	179	13.76
Gentamycin (well diffusion method)	316	24.30
Gentamycin. (disc diffusion method)	299	23
Chloramphenicol. (well diffusion method)	314	24.15
Chloramphenicol. (disc diffusion method)	317	24.38
Ciprofloxacin. (well diffusion method)	374	28.76
Ciprofloxacin. (disc diffusion method)	372	28.61

Graph-8

Mean zone of inhibition in the antibacterial sensitivity tests done in the Aravind Eye Hospital, Madurai, India.



CG = chlorhexidine gluconate

CA = chlorhexidine acetate

Gen WD = gentamicin well diffusion method

Gen DD = gentamicin disc diffusion method

Clo WD = chloramphenicol well diffusion method

Clo DD = chloramphenicol disc diffusion method

Cip WD = ciprofloxacin well diffusion method

Cip DD = ciprofloxacin disc diffusion method

In the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh, out of 71 patients there was no growth in 4 patients, no scrapings were available for culture in 4 patients and sensitivity test was done against 63 isolates and are presented in Appendix-13. The summarised results are shown in Table-8 and Graphs-9 and 10. The fungal isolates included *Fusarium sp.* 22 (34.92%), *Aspergillus sp.* 22 (34.92%), *Curvularia sp.* 4 (6.34%), *Cylindrocarpon sp.* 4 (6.34%), *Drechslera sp.* 1 (1.58%), and unidentified 10 (15.87%) and are shown in Table-9.

Table-8

Summary of results of antifungal sensitivity tests in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Name of drugs	Total no. of fungi tested	No. of positive responses	No response (no zone of inhibition)	Total zone of inhibition in mm	Mean zone of inhibition in positive cases in mm
0.2% chlorhexidine	61	54 (88.52%)	07	543	10.05
2.5% natamycin	61	44 (72.13%)	17	629	14.29
1% econazole	61	54 (88.52%)	07	830	15.37

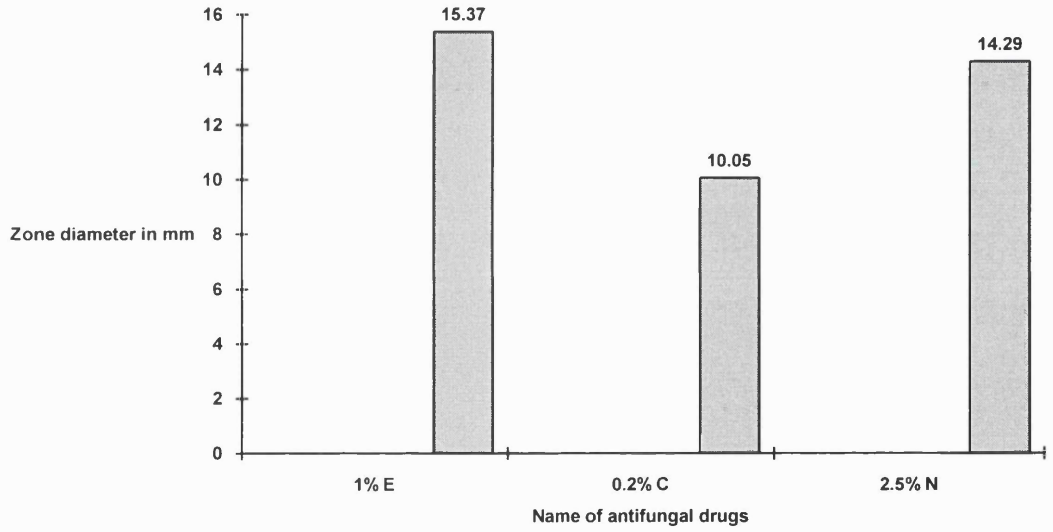
Table-9

Corneal isolates in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

			Drugs used	
Name of micro-organisms	Total no. of fungi	Percentage	0.2% chlorhexidine	2.5% natamycin
Fusarium sp.	22	34.92%	10	12
Aspergillus sp.	22	34.92%	09	13
Curvularia sp.	05	7.93%	04	01
Lasiodiplodia theobromae	04	6.34%	02	02
Coelomycete	01	1.58%	00	01
Colletotrichum dematium	01	1.58%	00	01
Cylindrocarpon sp..	03	4.76%	02	01
Drechslera sp.	01	1.58%	00	01
Unidentified fungi	04	6.34%%	03	01

Graph-9

Mean zone of inhibition in positive responses of antifungal sensitivity tests in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.



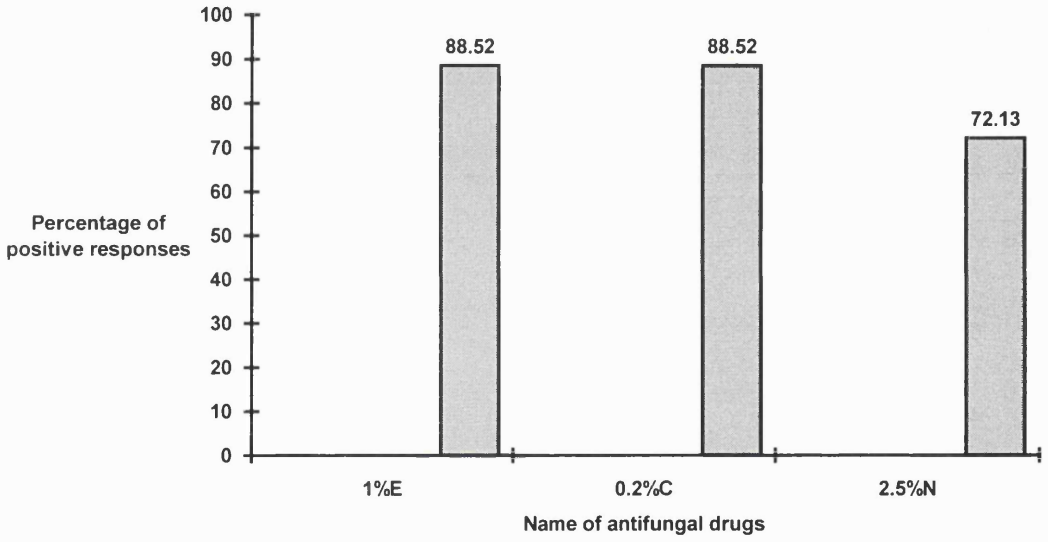
C = chlorhexidine

E = econazole

N = natamycin

Graph-10

Percentage of positive responses of antifungal sensitivity tests in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.



C = chlorhexidine

E = econazole

N = natamycin

5.2. Pilot study of treatment

24 patients were studied. The results are shown in the Table-10 and Graph-11. Five patients were treated with povidone iodine 5%, none were classified as severe and treatment failure occurred in four out of five patients. Of these 4 patients we changed to treatment of 2 patients with 0.2% chlorhexidine and 2 patients with 1% econazole and all were healed. Povidone iodine was therefore discontinued. Nine patients were treated with econazole 1%, there was one severe case which did not respond, non-severe cases were healed and one patient absconded leaving 8 patients in the study. The causative micro-organism in the severe one was *Aspergillus flavus* . In the chlorhexidine group (11 cases), three severe cases and all non-severe cases were healed. One severe case failed to respond. It was a sloughing corneal ulcer measuring about 7 mm. We treated the eye for 5 days then changed to 1% econazole but there was no improvement and ultimately the eye was perforated. The causative fungus was again *Aspergillus flavus*.

The characteristics of these patients are shown in Tables-11 to 19. The right eye was affected 2 times more than the left eye (Table-11). The ratio of affected male and female was 2:1 (Table-12). About 75% of affected patients were 31 to 50 years of age, of them 50% was 31 to 40 years and 25% was 41 to 50 years age group (Table-13). Farmers were mainly affected, about 59% (Table-14). 66.66% had a history of trauma (Table-15). 33.33% had mixed treatment before coming to hospital, 20.83% antibiotic and 12.50% native medicines (Table-16). 41.66% of patients attended hospital in the 1st week and 33.33% in the 2nd week (Table-17). Visual acuities were recorded prior to corneal scraping. 17% patients presented with perception of light and 17% patients presented with hand movement and are shown in Table-18.

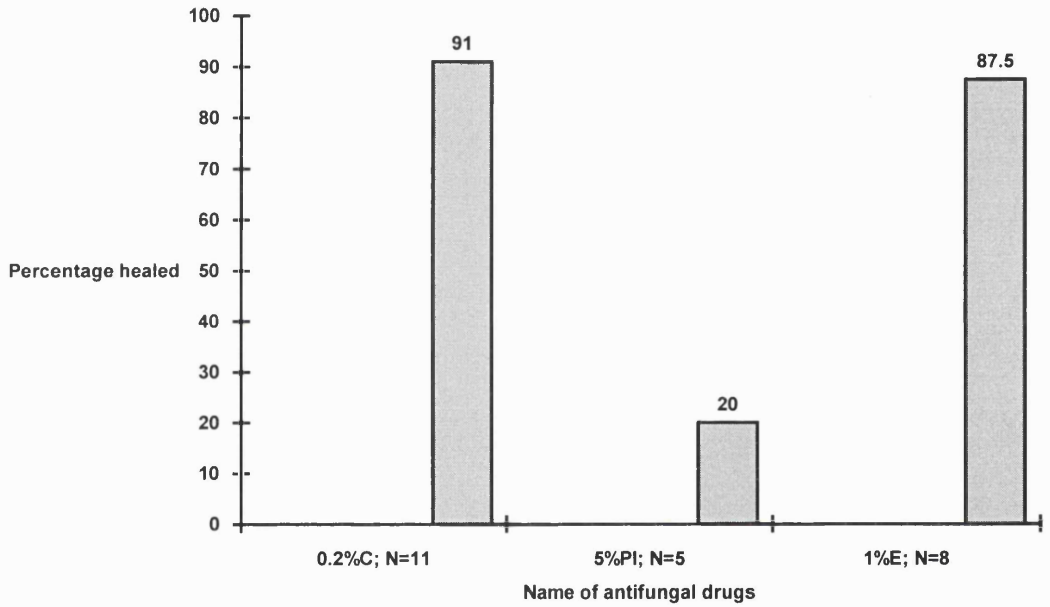
Table-10

Results at 14 days of the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

		Severe		Non-severe		
Treatment group (Name of drugs)	Total No. of patients	Healed	No response	Healed	No response	Total healed
0.2% chlorhexidine	11	03 (75%)	01 (25%)	07	00	10 (91%)
5% povidone iodine	05	00	00	01	04	01 (20%)
1% econazole	08	00	01 (100%)	07	00	07 (87.5%)

Graph-11

Percentage of healed fungal keratitis in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.



C = chlorhexidine

PI = povidone iodine

E = econazole

N = total number of cases.

Table-11

Eye affected in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

Affected eye	0.2% chlorhexidine	5% povidone iodine	1% econazole	Total number	Percentage
Right eye	06	04	06	16	66.66%
Left eye	05	01	02	08	33.33%

Table-12

Sex distribution of patients in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

Sex	0.2% chlorhexidine	5% povidone iodine	1% econazole	Total no. of patients	Percentage
Male	10	02	04	16	66.66%
Female	01	03	04	08	33.33%

Table-13

Age distribution of patients in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

Age group (Years)	0.2% chlorhexidine	5% povidone iodine	1% econazole	Total no. of patients	Percentage
0-10	00	00	00	00	0%
11-20	01	00	01	02	8.33%
21-30	01	01	00	02	8.33%
31-40	05	03	04	12	50%
41-50	02	01	03	06	25%
51-60	02	00	00	02	8.33%

Table-14

Occupation of patients in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

Occupation	0.2% chlorhexidine	5% povidone iodine	1% econazole	Total number	Percentage
Farmer	06	03	05	14	58.33%
House wife	01	02	00	03	12.50%
Service	01	00	00	01	4.16%
Student	01	00	01	02	8.33%
Weaver	01	00	00	01	4.16%
Milk man	01	00	00	01	4.16%
Business man	00	00	01	01	4.16%
Labourer	00	00	01	01	4.16%

Table-15

Predisposing factors of fungal keratitis in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

Predisposing factors		0.2% chlorhexidine	5% povidone iodine	1% econazole	Total number	Percentage
Trauma		05	04	07	16	66.66%
	Stick	01	00	01	02	
	Paddy grain	01	01	00	02	
	Finger	01	00	00	01	
	Stone	00	01	00	01	
	Plant	00	00	01	01	
	Thorn	00	00	01	01	
	Dust	00	00	01	01	
	Lemon juice	00	00	01	01	
	Heat	00	00	01	01	
	Unknown foreign body	02	02	01	05	
No history of trauma		06	01	01	08	33.33%

Table-16

Medications prior to corneal scrapings in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

Medicines used	0.2% chlorhexidine	5% povidone iodine	1% econazole	Total number	Percentage
Antibiotics	04	00	01	05	20.83%
Antifungals	01	00	00	01	4.16%
Antivirals	00	00	00	00	0%
Steroids	00	00	00	00	0%
Eye drops (Unknown)	02	01	01	04	16.66%
Native medicines	01	00	02	03	12.50%
Mixed	03	03	02	08	33.33%
None	00	01	02	03	12.50%

Table-17

Duration of illness prior to corneal scrapings in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

Duration (Days)	0.2% chlorhexidine	5% povidone iodine	1% econazole	Total number of patients	Percentage
1 to 7	05	03	02	10	41.66%
8 to 14	03	02	03	08	33.33%
15 to 21	01	00	01	02	8.33%
22 to 30	00	00	02	02	8.33%
31 to 60	02	00	00	02	8.33%

Table-18

Visual acuity of patients prior to corneal scrapings in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

Visual acuity	0.2% chlorhexidine	5% povidone iodine	1% econazole	Total number of patients	Percentage
6/6 (P)	01	00	01	02	8.33%
6/12	03	00	01	04	16.66%
6/18	00	01	00	01	4.16%
6/60	00	00	01	01	4.16%
6/36	00	01	03	04	16.66%
3/60	01	00	01	02	8.33%
1/60	01	00	00	01	4.16%
C.F.	00	01	00	01	4.16%
H.M.	03	01	00	04	16.66%
P.L.	02	01	01	04	16.66%

Table-19

Results of Gram stain and potassium hydroxide (KOH) tests in the pilot study of treatment done in the Aravind Eye Hospital, Madurai, India.

Name of tests	0.2% chlorhexidine	5% povidone iodine	1% econazole	Total number	Percentage
Positive with Gram.	04	02	03	09	37.50%
Negative with Gram	07	03	05	15	62.50%
KOH positive	11	05	08	24	100%
KOH negative	00	00	00	00	0%

5.3. Randomised trial of different concentrations of chlorhexidine and natamycin 5%.

60 patients were studied and the results are shown in the Table-20 and Graphs 12 and 13. Two patients were lost to follow-up, and 12 were classified as severe with little prospect of recovery, shown in Table-21. Statistical analysis is shown in the Tables 22 to 25 and Graphs 14 to 16. At 5 days the response was related to the concentration of chlorhexidine, with 0.2% giving the best results. The superiority of 0.2% chlorhexidine over natamycin 5% was statistically significant in patients not having had prior antifungal treatment.

Out of 60 patients there were 12 severe corneal ulcers and none of them were healed by chlorhexidine or by natamycin. The overall healing rates were 62.50% and 43.75% by 0.2% chlorhexidine and 5% natamycin respectively. In the case of non-severe ulcers the healing rates were 83.33% and 50% by 0.2% chlorhexidine and 5% natamycin respectively, Table-20 and Graphs 12 and 13. The 4 treatments are compared in respect of outcome at 5 days in Table-22 and Graphs 14 to 16. When the data are stratified by severity of ulcers, it can be seen that the subgroup of patients with severe ulcers (Stratum-2) do not contribute to the comparative analysis, because only one in this subgroup has a favourable outcome Graph-16. Furthermore, none of this subgroup were cured within 21 days. Therefore, in all subsequent analyses, the 12 patients with severe ulcers are excluded, leaving 46 patients for analysis (Tables 23 to 25).

In Table 23 the frequency of various patient characteristics that may be potentially prognostic are shown for each treatment group. There are a number of characteristics that are distributed unequitably between the treatment groups. The data show that when patients are grouped (Stratified) according to presence or absence of the characteristics, the resulting subgroups are too small to allow detailed stratified analysis, whereby adjustments could have been made for possible confounding effects of the prognostic factors. For example, 21.4% of the patients in the natamycin group have had prior antifungal treatment, whereas none of the patients in the chlorhexidine 0.2% group had prior treatment with antifungal drugs.

Further analyses are therefore limited to unstratified data, together with analysis in a few of the important strata (Subgroups) that have sufficient numbers. This helps to remove some of the possible confounding. For the same reasons (small numbers), multivariable regression analyses were not done.

Analysis of all 46 patients (unstratified by confounders) suggests that chlorhexidine 0.2% may be superior to natamycin, in producing a favourable response at 5 days. The difference in efficacy between the two treatments is of borderline significance (exact p=0.051). (Table-24).

When 5 patients who had previous antfungal treatment are excluded, a superiority of chlorhexidine 0.2% over natamycin becomes convincing, and is statistically significant (exact p=0.043)-as shown in Stratum-1 in Table-24. Stratum-2 is not useful as there are too few patients in it.

The dose-dependent trend in both Tables 22 and 24 is worthy of note. The 4 treatments are also compared in respect of efficacy to produce cure within 21 days (Table-25). Chlorhexidine at 0.2% concentration appears superior compared with lower concentrations, and compared with natamycin 5%. The differences, however, are not statistically significant. Three patients who had shown a favourable response at 5 days continued to improve on the same treatment but were not completely healed at 21 days. Further analyses of subgroups, formed by levels of possible prognostic variables, do not lead to significant differences.

The superiority of chlorhexidine 0.2% in the study sample is apparent in males and females, in younger and older age groups, and in subgroups formed by duration of ulcer. The non-significant differences should be interpreted with due consideration of the power of the study. No toxic effects were experienced by any of the treatment groups.

The characteristics of the patients are shown in Tables-26 to 36. Right eyes were more affected than left eyes (Table-26). Male and female were affected in the percentage of 71.66% and 28.33% respectively (Table-27). Corneal ulcer mostly occurred in the 31 to 50 age group as was in the pilot study of treatment and accounts for about 50% of total patients (Table-28). About 57% of affected people were farmers, others were out door working groups i.e. those who are very much prone to trauma (Table-29). Trauma was the main predisposing factor for fungal keratitis, occuring in about 54% (Table-30). Patients used drugs indiscriminately. They have mainly used antibiotics. Some patients have used native medicines, steroids and antivirals and are shown in Table-31. Some of them had used native medicine, steroids, antibiotics etc. in the same eye. About 48% patients attended cornea clinic with in the 1st week of illness and 35% patients by the 2nd week of illness, as shown in Table-32. Visual acuities

were recorded prior to corneal scraping; about 31.6% patients presented with counting fingers and 25% patients presented with hand movement, as shown in Table-33. About 79% of the patients were from rural areas (Table-34). The literate people were more affected than the illiterate in the percentage 55% and 45% respectively (Table-35). Those who can at least write their names are considered to be literate here.

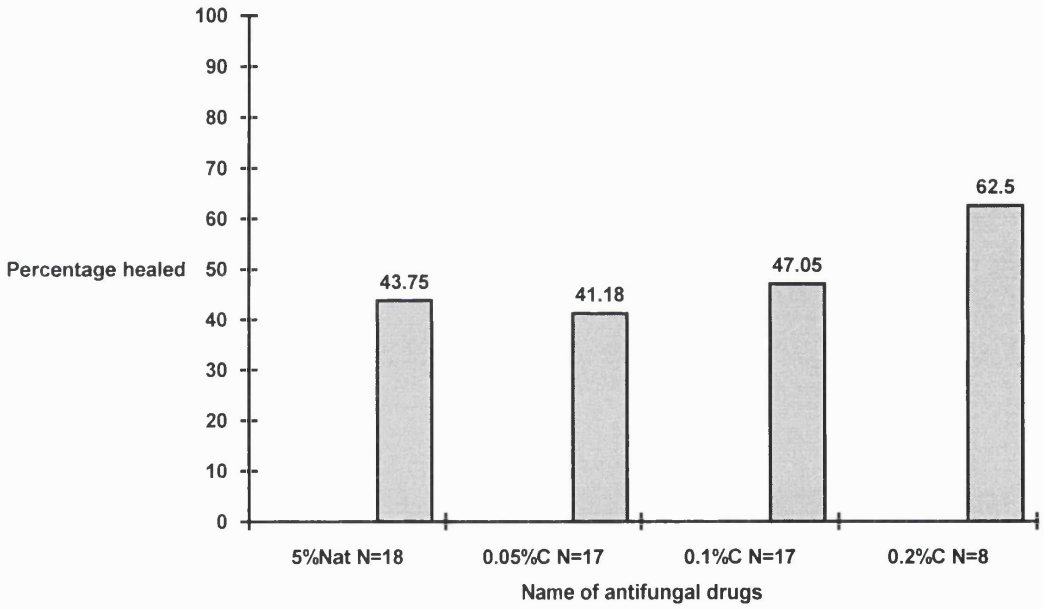
Table-20

Results of antifungal drugs in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Name of drugs	No. of patients	Ulcers healed by 21 days	No response	Percentage healed in non-severe ulcers only	Not healed by 3 week
0.05% chlorhexidine	17	7 (41.18%)	09	58.33%	01
0.1% chlorhexidine	17	8 (47.05%)	07	57.14%	02
0.2% chlorhexidine	08	5 (62.50%)	02	83.33%	01
5% natamycin	16	7 (43.75%)	09	50%	00

Graph-12

Percentage of healed fungal keratitis in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.



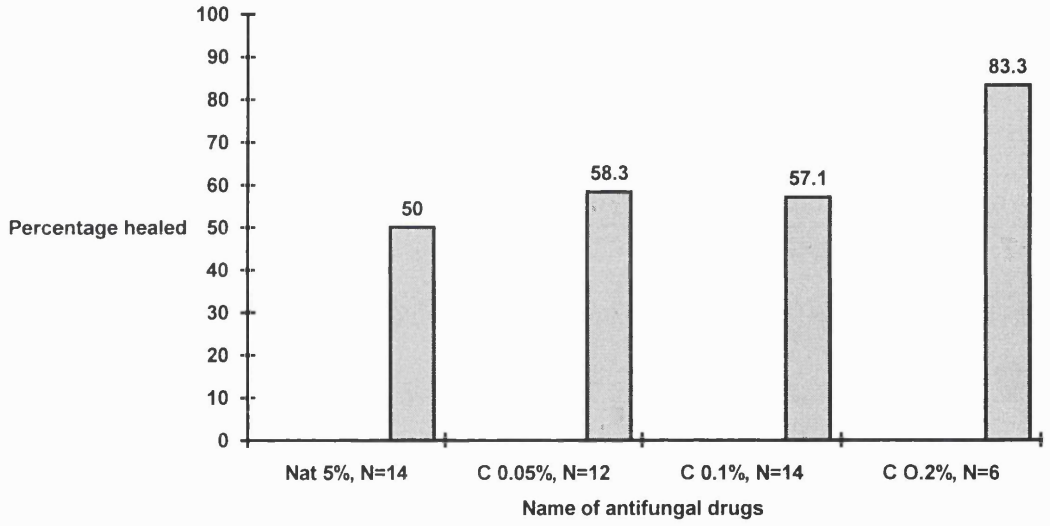
C = chlorhexidine

Nat = natamycin

N = total number of patients

Graph-13

Percentage of healed non-severe fungal keratitis in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.



C = chlorhexidine

Nat = natamycin

N = total number of patients

Table-21

Patients randomised to 4 treatment groups in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the AEH, Madurai, India.

Treatment group	Not severe	Severe	Total
Chlorhexidine 0.05%	12	05	17
Chlorhexidine 0.1%	14	03	17
Chlorhexidine 0.2%	06	02	08
Natamycin 5%	16**	02	18
Total	48	12	60

** Two patients were lost to follow up leaving 58 patients in the study.

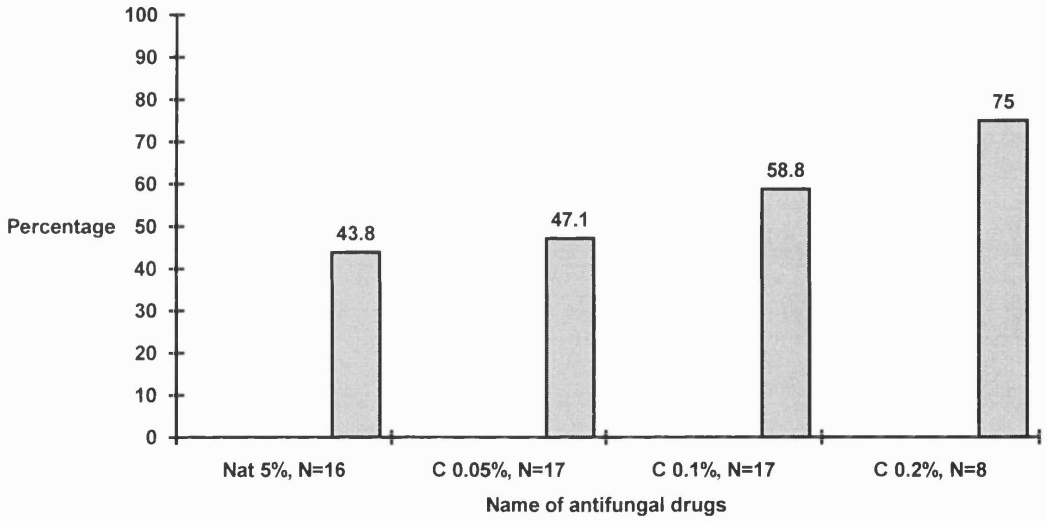
Table-22

Outcome of treatment at 5 days, stratified by severity of ulcers in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

	No. of patients	Favourable response	Percentage	No response	Percentage
ALL ULCERS					
Chlorhexidine 0.05%	17	08	47.1%	09	52.9%
Chlorhexidine 0.1%	17	10	58.8%	07	41.2%
Chlorhexidine 0.2%	08	06	75%	02	25%
Natamycin 5%	16	07	43.8%	09	56.3%
Total	58	31		27	
Stratum 1-ULCERS NOT SEVERE					
Chlorhexidine 0.05%	12	07	58.3%	05	41.7%
Chlorhexidine 0.1%	14	10	71.4%	04	28.6%
Chlorhexidine 0.2%	06	06	100%	00	0%
Natamycin 5%	14	07	50%	07	50%
Total	46	30		16	
Stratum 2-ULCERS SEVERE					
Chlorhexidine 0.05%	05	01	20%	04	80%
Chlorhexidine 0.1%	03	00		03	100%
Chlorhexidine 0.2%	02	00		02	100%
Natamycin 5%	02	00		02	100%
Total	12	01		11	

Graph-14

Outcome of favourable response at 5 days, in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.



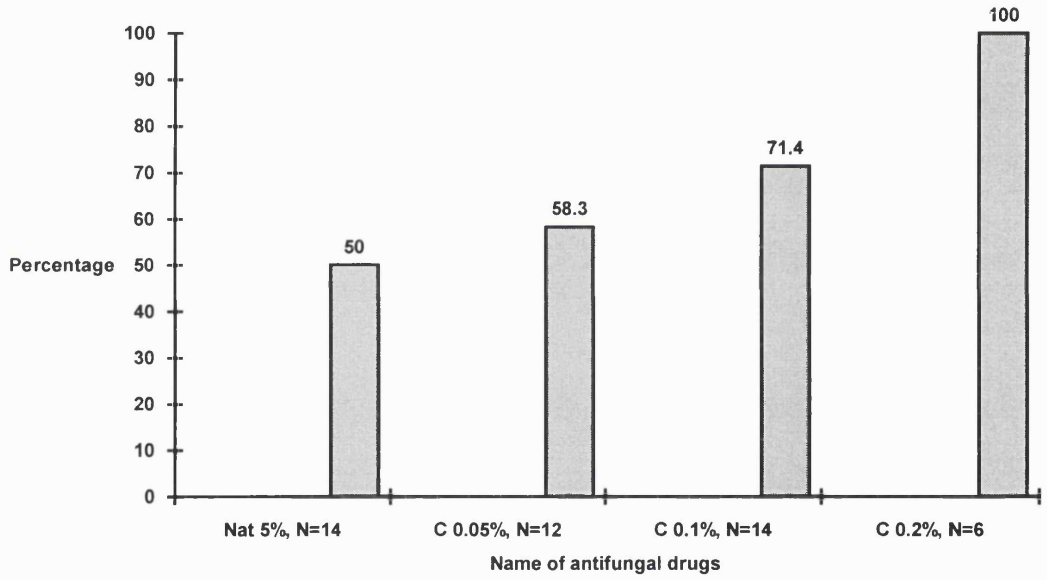
C = chlorhexidine

Nat = natamycin

N = number of patients

Graph-15

Outcome of favourable response at 5 days only in the ulcers not severe, in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.



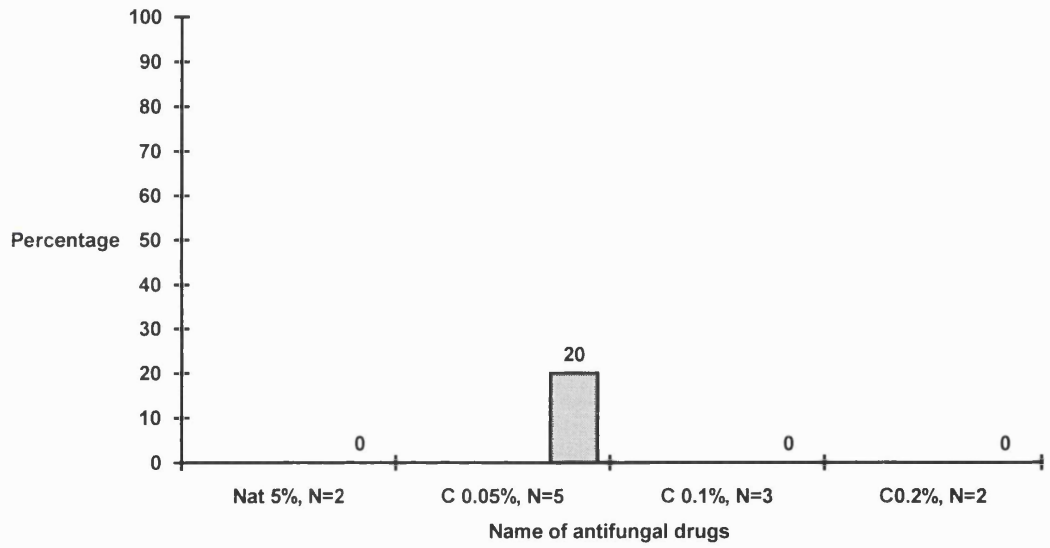
C = chlorhexidine

Nat = natamycin

N = number of patients

Graph-16

Outcome of favourable response at 5 days only in the severe ulcers, in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.



C = chlorhexidine

Nat = natamycin

N = number of patients

Table-23

Baseline characteristics of 46 patients in the 4 treatment groups in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

(12 patients with severe ulcers and 2 patients lost to follow-up excluded)

Characteristic at randomisation		Chlhx 0.05%		Chlhx 0.1%		Chlhx 0.2%		Natamycin 5%		All patients	
		n	%	n	%	n	%	n	%	n	%
Age	0-49 years	8	66.7	12	85.7	3	50	8	57.1	31	67.4
	>49 years	4	33.3	2	14.3	3	50	6	42.9	15	32.6
Sex	Male	9	75	9	64.3	5	83.3	12	85.7	35	76.1
	Female	3	25	5	35.7	1	16.7	2	14.3	11	23.9
Literacy	Not literate	3	25	6	42.9	4	66.7	4	28.6	17	37
	Literate	9	75	8	57.1	2	33.3	10	71.4	29	63
Home	Rural area	9	75	9	64.3	6	100	12	85.7	36	78.3
	Urban area	3	25	5	35.7	0	00	2	14.3	10	21.7
Trauma: Positive history		6	50	7	50	2	33.3	9	64.3	24	52.2
*Prior treatment	None	8	66.7	12	85.7	5	83.3	11	78.6	36	78.3
	Antibiotics	3	25	4	28.6	4	66.7	2	14.3	13	28.3
	Steroids	0	00	1	7.2	0	00	0	0.0	1	2.2
	Antifungals	2	16.7	0	00	0	00	3	21.4	5	10.9
	Antivirals	2	16.7	2	14.3	0	00	1	7.1	5	10.9
	Others	2	16.7	7	50	1	16.7	6	42.9	16	34.8
Duration of ulcer	1-9 days	7	58.3	8	57.1	2	33.3	8	57.1	25	54.3
	>9 days	5	41.7	6	42.9	4	66.7	6	42.9	21	45.7
Mean age		42.6		36.4		45.7		44.3		41.6	
Standard deviation		16.2		13.2		13.4		17.3		15.3	
Mean duration (days)		7.3		8.6		23.2		10.2		10.7	
Standard deviation		4.1		4.8		21.6		7.8		10.3	

* These are not exclusive, as a patient may have more than one prior treatment.

Table-24

Outcome of treatment at 5 days, unstratified and stratified by prior antifungal treatment in the randomised trial of different concentrations of chlorhexidine natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

(12 patients with severe ulcers are excluded from this analysis)

Outcome of treatment at 5 days

	No. of patients	Favourable response	Relative efficacy (natamycin as referent)	* Exact test 2-sided p-value
UNSTRATIFIED (All patients)				
Chlorhexidine 0.05%	12	7 (58.3%)	1.17	>0.1
Chlorhexidine 0.1%	14	10 (71.4%)	1.43	>0.1
Chlorhexidine 0.2%	06	6 (100%)	2.00	0.051
Natamycin 5%	14	7 (50%)	1 (referent)	
Total	46	30		
STRATIFIED BY PRIOR ANTIFUNGAL TREATMENT				
Stratum 1-No prior antifungal treatment:				
Chlorhexidine 0.05%	10	5 (50%)	1.10	>0.1
Chlorhexidine 0.1%	14	10 (71.4%)	1.57	>0.1
Chlorhexidine 0.2%	06	6 (100%)	2.20	0.043
Natamycin 5%	11	5 (45.5%)	1 (referent)	
Total	41	26		
Stratum 2-Had prior antifungal treatment:				
Chlorhexidine 0.05%	02	02 (100%)	1.50	>0.1
Chlorhexidine 0.1%	00	00		
Chlorhexidine 0.2%	00	00		
Natamycin 5%	03	02 (66.7%)	1 (referent)	
Total	05	04		

*Fishers Exact Test, comparing efficacy of each treatment with the referent natamycin.

Table-25

Outcome of treatment at 21 days, in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

(12 patients with severe ulcers excluded are excluded from this analysis)

Outcome of treatment at 21 days

	Number of patients	Cured on original treatment within 21 days	Relative efficacy (natamycin as referent)	* Exact test 2-sided p-value
UNSTRATIFIED (all patients)				
Chlorhexidine 0.05%	12	7 (58.3%)	1.17	> 0·1
Chlorhexidine 0.1%	14	8 (57.1%)	1.14	> 0·1
Chlorhexidine 0.2%	06	5 (83.3%)	1.67	> 0·1
Natamycin 5%	14	7 (50%)	1 (referent)	
Total	46	27		

*Fishers Exact Test, comparing efficacy of each treatment with the referent natamycin.

Table-26

Eye affected in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Eye	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	Total number	Percentage
Right eye	09	09	06	11	35	58.33%
Left eye	08	08	02	07	25	41.66%

Table-27

Sex distribution of patients in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Sex	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	Total no. of patients	Percentage
Male	11	10	06	16	43	71.66%
Female	06	07	02	02	17	28.33%

Table-28

Age distribution of patients in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Age (Years)	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	Total number	Percentage
0-10	00	01	00	00	01	1.66%
11-20	01	01	00	01	03	5%
21-30	02	02	02	03	09	15%
31-40	02	07	02	04	15	25%
41-50	07	03	02	03	15	25%
51-60	03	02	03	05	13	21.66%
61-70	01	00	00	02	03	5%
71-80	01	00	00	00	01	1.66%

Table-29

Occupation of patients in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Occupations	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	No. of patients	Percentage
Farmer	11	05	07	11	34	56.66%
Coolie	00	02	01	03	06	10%
Labourer	00	01	00	02	03	5%
Businessman	00	03	00	00	03	5%
Student	01	02	00	00	03	5%
Watch man	01	01	00	00	02	3.33%
House wife	00	01	00	00	01	1.66%
Hawker	01	00	00	00	01	1.66%
Sugar mill worker	00	01	00	00	01	1.66%
Fisher man	01	00	00	00	01	1.66%
Driver	00	00	00	01	01	1.66%
Weaver	00	01	00	00	01	1.66%
Post man	01	00	00	00	01	1.66%
Rickshaw puller	01	00	00	00	01	1.66%
Silver smith	00	00	00	01	01	1.66%

Table-30

Predisposing factors of fungal keratitis in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Predisposing factors		0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	No. of patients	Percentage
Trauma		08	08	03	13	32	53.33%
	Foreign body (Unknown)	04	02	00	03		
	Stick	00	02	02	05		
	Dust	00	01	00	01		
	Sugar cane leaf	00	01	00	01		
	Iron	00	00	01	00		
	Stone	01	00	00	00		
	Cloth	01	00	00	00		
	Thorn	00	01	00	00		
	Seed	01	00	00	00		
	Wood	00	01	00	00		
	Rice grain	00	00	00	01		
	Lime	00	00	00	01		
	Insect	00	00	00	01		
	Sand	01	00	00	00		
No history of trauma		08	09	05	04	26	43.33%
Hansen's disease		01	00	00	01	02	3.33%

Table-31

Medications prior to corneal scraping in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Name of drugs	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	No. of patients	Percentage
Antibiotics	04	04	04	04	16	26.66%
Steroids	00	01	00	01	02	3.33%
Antifungals	01	00	00	01	02	3.33%
Antivirals	02	00	00	01	03	5%
Native medicine	00	00	01	02	03	5%
Eye drops (Unknown)	04	05	01	05	15	25%
None	05	02	01	03	11	18.33%
Mixed	01	05	01	01	08	13.33%

Table-32

Duration of illness prior to corneal scraping in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Duration (Days)	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	No. of patients	Percentage
1 to 7	07	09	03	10	29	48.33%
8 to 14	08	05	01	05	19	35%
15 to 21	02	03	01	02	08	10%
22 to 30	00	00	02	01	03	5%
31 to 60	00	00	01	00	01	1.66%

Table-33

Visual acuity of patients prior to corneal scraping in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Visual acuity	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	No. of patients	Percentage
6/12	01	02	00	00	03	5%
6/18	00	01	00	02	03	5%
6/24	02	01	00	01	04	6.66%
6/36	02	01	00	03	06	10%
6/60	00	00	01	01	02	3.33%
C.F.	03	04	04	08	19	31.66%
H.M.	07	05	01	02	15	25%
P.L.	02	03	02	01	08	13.33%

Table-34

Location of patients in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Location	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	Total number	Percentage
Rural	14	11	08	14	47	78.33%
Urban	03	06	00	04	13	21.66%

Table-35

Literacy of patients in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Literacy	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	Total number	Percentage
Literate	09	09	03	12	33	55%
Illiterate	08	08	05	06	27	45%

Table-36

Results of Gram stain and potassium hydroxide (KOH) tests in the randomised trial of different concentrations of chlorhexidine and natamycin 5% done in the Aravind Eye Hospital, Madurai, India.

Name of tests	0.05% chlorhexidine	0.1% chlorhexidine	0.2% chlorhexidine	5% natamycin	Total number	Percentage
Positive with Gram.	14	12	07	12	45	75%
Negative with Gram.	03	05	01	06	15	25%
KOH positive	17	17	08	18	60	100%
KOH negative	00	00	00	00	00	0%

5.4. Randomised trial of chlorhexidine 0.2% and natamycin 2.5%

We studied 71 patients, of which 35 were allocated to the chlorhexidine 0.2% group and 36 to the natamycin 2.5% group (Table 37). Twenty patients were classified as severe, with little prospect of recovery (although 3 severe ulcers were in fact healed beyond 21 days by chlorhexidine 0.2%).

The baseline characteristics of the patients and details of the ulcers are shown in Tables 38 and 39. The categories used in the stratified analyses are given in Table 40. Other characteristics of the ulcers and of the two treatment groups are given in Tables 48 to 58.

Analysis of favourable outcomes of all 71 patients at 5 days suggests that chlorhexidine 0.2% may be superior to natamycin 2.5%. The favourable outcomes at day 5 were 88.6% and 52.8% with chlorhexidine 0.2% and natamycin 2.5% respectively, the relative efficacy being 1.68 [CI 1.21-2.34], as shown in Table 41. The stratified analysis of patients characteristics and ulcer details are shown in Table 42. If we consider the patients who had an ulcer size more than 12 sq. mm, a superiority of chlorhexidine 0.2% over natamycin 2.5% in producing a favourable response at 5 days becomes more convincing, and statistically significant. Favourable responses were 92.9% and 33.3% with chlorhexidine 0.2% and natamycin 2.5% respectively, shown in Table 43. The relative efficacy was 2.79 [CI 1.5 - 5.19]. These results are shown graphically in Graphs 17 to 19.

In the chlorhexidine 0.2% group 1 non-severe ulcer dropped out after 7 days for follow up, 1 severe and 1 non-severe ulcer were improving at 5 days but the study was concluded before 21 days, leaving 32 in the study. In the natamycin 2.5% group 1 non-severe ulcer dropped out after 7 days for follow up, 1 non-severe and 1 severe ulcers were improving at 5 days but the study was concluded before 21 days, leaving 33 in the study, altogether 65 patients at 21 days.

None of the severe ulcers were healed either by chlorhexidine 0.2% or natamycin 2.5% by 21 days. Three severe ulcers were healed beyond 21 days by chlorhexidine 0.2%, 1 after 26 days, 1 after 37 days, and 1 after 60 days, but none by natamycin 2.5%. The overall healing rates at 21 days were 43.8% and 30.3% by chlorhexidine 0.2% and natamycin 2.5% respectively. In the case of non-severe ulcers the healing rates were 66.66% and 38.46% by chlorhexidine 0.2% and natamycin 2.5%

respectively. The relative efficacy was 1.44, as shown in the Tables 44, 45 and Graphs 20, 21. Adjustment for confounding effect of other prognostic factors makes little or no differences to the findings (Table 46).

Out of 71 patients we could follow up 65 patients to see the ultimate fate of the ulcers. 24 healed by 21 days (Table-45) and 41 had no response or a favourable response by 5 days, of which 3 severe ulcers healed beyond 21 days by 0.2% chlorhexidine, 12 healed by alternative treatment, 1 was still under alternate treatment, 10 had anterior staphyloma, 9 adherent leucoma, 2 got penetrating keratoplasty, 3 were enucleated and 1 eviscerated (Table-47).

The organisms isolated from the ulcers in the two treatment groups are listed in Table 48. The right eye was more affected than the left eye (Table-49). Both male and female were affected in the percentage of 74.64% and 25.35% respectively (Table-50). Corneal ulcer mostly occurred in the 21 to 50 age group, which accounts about 64.77% of total patients (Table-51). About 35% affected people were farmers, others were outdoor working groups i.e. those who are very much prone to trauma (Table-52). Trauma was the main predisposing factor for fungal keratitis, accounting for about 56% (Table-53). Some patients had used steroids, antibiotics etc. in the same eye. Some of them had used native medicines, even snail juice and are shown in Table-54. About 21.12% patients attended the cornea clinic within the 1st week of illness and 35% patients by the 2nd week of illness, as shown in Table-55. Visual acuities were recorded prior to corneal scraping. About 32% patients presented with hand movement, as shown in Table-56. Visual acuities after healing are shown in Table-57 & 58. The frequency of various patient characteristics were not statistically significant (Table-38). It is almost same for all the characteristics.

Gradual improvement or deterioration of fungal corneal ulcers and hypopyon are shown in Illustrations 2 to 9.

Table-37

Patients randomised to 2 treatment groups in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Treatment groups	Not severe	Severe	Total
Chlorhexidine 0.2%	23	12	35
Natamycin 2.5%	28	08	36
Total	51	20	71

Table-38

Baseline characteristics of the 2 treatment groups in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

	TREATMENT				Table Total	
	Chlorhexidine		Natamycin		Count	Col %
	Count	Col %	Count	Col %		
Age group						
10-39	08	22.9%	14	38.9%	22	31.0%
40-49	17	48.6%	14	38.9%	31	43.7%
50-75	10	28.6%	08	22.2%	18	25.4%
Sex						
Male	25	71.4%	28	77.8%	53	74.6%
Female	10	28.6%	08	22.2%	18	25.4%
Prior Antibiotic						
no	12	34.3%	15	41.7%	27	38.0%
yes	23	65.7%	21	58.3%	44	62.0%
Prior Treatment						
1- Antibiotic	22	62.9%	19	52.8%	41	57.7%
2- Steroid			03	8.3%	03	4.2%
3- Native med.	01	2.9%			01	1.4%
4- Other med.	11	31.4%	11	30.6%	22	31.0%
5- Antifungal			01	2.8%	01	1.4%
1- & 5-			02	5.6%	02	2.8%
1- 2- & 5-	01	2.9%			01	1.4%
Ulcer duration						
1- 10	16	45.7%	14	38.9%	30	42.3%
> 10 days	19	54.3%	22	61.1%	41	57.7%
Ulcer size						
1- 6 sq. mm	12	34.3%	06	16.7%	18	25.4%
6.1- 12 sq. mm	09	25.7%	09	25.0%	18	25.4%
12.1- 25 sq. mm	06	17.1%	12	33.3%	18	25.4%
> 25 sq. mm	08	22.9%	09	25.0%	17	23.9%
Epith. defect.						
A. Less than 2 mm	06	17.1%	02	5.6%	08	11.3%
B. 2 - 4 mm.	15	42.9%	15	41.7%	30	42.3%
C. 4 - 6 mm	07	20.0%	12	33.3%	19	26.8%
D. 6 - 8 mm.	07	20.0%	07	19.4%	14	19.7%

Table-38 (continued).

Baseline characteristics of the 2 treatment groups in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

	TREATMENT				Table Total	
					Count	Col %
	Chlorhexidine 0.2%		Natamycin 2.5%			
Ulcer depth						
A. Superficial 1/3	14	40.0%	18	50.0%	32	45.1%
B. Superficial 2/3	15	42.9%	16	44.4%	31	43.7%
C. Deep	06	17.1%	02	5.6%	08	11.3%
Hypopyon						
A. No	18	51.4%	15	41.7%	33	46.5%
B. 0 - 2 mm.	14	40.0%	16	44.4%	30	42.3%
C. 2 - 5 mm./	02	5.7%	04	11.1%	06	8.5%
D. More than 5 mm.	01	2.9%	01	2.8%	02	2.8%
Infiltrate						
A. 0 -3o%	10	28.6%	08	22.2%	18	25.4%
B. 30 -60%	21	60.0%	26	72.2%	47	66.2%
C. 60 -100%	04	11.4%	02	5.6%	06	8.5%
Deep lesion						
A. None	29	82.9%	36	100.0%	65	91.5%
B. Posterior corneal abscess	03	8.6%			03	4.2%
C. Endothelial plaque	03	8.6%			03	4.2%
Severe ulcer	12	34.3%	08	22.2%	20	28.2%
Perforation threat	09	25.7%	06	16.7%	15	21.1%
Table Total	35	100.0%	36	100.0%	71	100.0%

Table-39

Baseline characteristics of the 2 treatment groups in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh, continuous variables

	TREATMENT	
	Chlorhexidine 0.2%	Natamycin 2.5%
AGE		
Mean	40.2	37.0
Minimum	15.0	10.0
Maximum	75.0	70.0
Std Deviation	15.2	15.6
Ulcer Duration (days)		
Mean	14.5	14.8
Minimum	3.0	4.0
Maximum	45.0	30.0
Std Deviation	9.4	7.2
Ulcer Size (sq. mm)		
Mean	17.5	19.9
Minimum	2.1	1.0
Maximum	72.0	72.0
Std. Deviation	18.8	15.8

Table-40

Categories used in the stratified analysis of results of the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Age	10-39 40-49 50-75
Sex	Male Female
Duration of ulcer	1-10 days > 10 days
Ulcer size	1-12 sq. mm > 12 sq. mm
Prior antibiotic	no yes
Severity of ulcer	not severe severe
All others	as shown in Table 38 for Baseline Characteristics

Table-41

Comparing the efficacy of 2 treatment groups at 5 days, with no adjustment for confounding in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Treatment group.	Number of patients	Efficacy % with favourable outcome at day 5	Relative Efficacy (RE) (chlorh./natam.)	95% con. limits for RE	P- value
Chlorhexidine 0.2%	35	88.6	1.68	1.21 - 2.34	0.001
Natamycin 2.5%	36	52.8			

Table-42

Comparing the relative efficacy of the 2 treatment groups in producing favourable outcome at day 5, after adjustment for confounding in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Factors adjusted for	Relative Efficacy (RE) of the 2 treatments. (chlorh./natam.)	95% conf. limits for RE	p- value
Age	1.76	1.25 - 2.47	< 0.001
Sex	1.68	1.20 - 2.35	0.001
Duration of ulcer	1.65	1.19 - 2.29	0.001
Prior antibiotic treatment	1.70	1.21 - 2.37	< 0.001
Severity of ulcer	1.76	1.22 - 2.54	< 0.001
Ulcer size	1.56	1.16 - 2.11	0.003
Degree of epithelial defect	1.71	1.19 - 2.46	0.003
Depth of ulcer	1.77	1.24 - 2.52	< 0.001
Degree of hypopyon	1.62	1.19 - 2.20	0.001
Degree of infiltrate	1.74	1.23 - 2.45	< 0.001

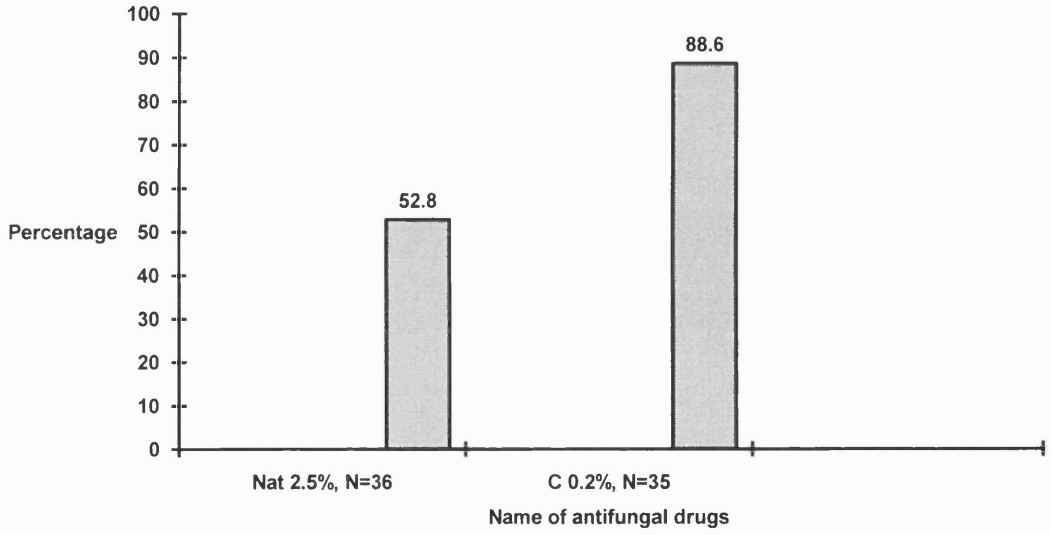
Table-43

Comparing the relative efficacy of the 2 treatment groups in producing favourable outcome at day 5, stratified by severity of ulcers in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Ulcer size stratum	Treatment group	Number of patients	Efficacy % with favourable outcome at day 5	Relative Efficacy (RE) (chlorh./natam.)	95% conf. limits for RE
1 - 12 sq. mm.	Chlorhexidine 0.2%	21	85.7	1.07	0.79 - 1.46
	Natamycin 2.5%	15	80.0		
> 12 sq. mm.	Chlorhexidine 0.2%	14	92.9	2.79	1.50 - 5.19
	Natamycin 2.5%	21	33.3		

Graph-17

Outcome of favourable response at 5 days, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.



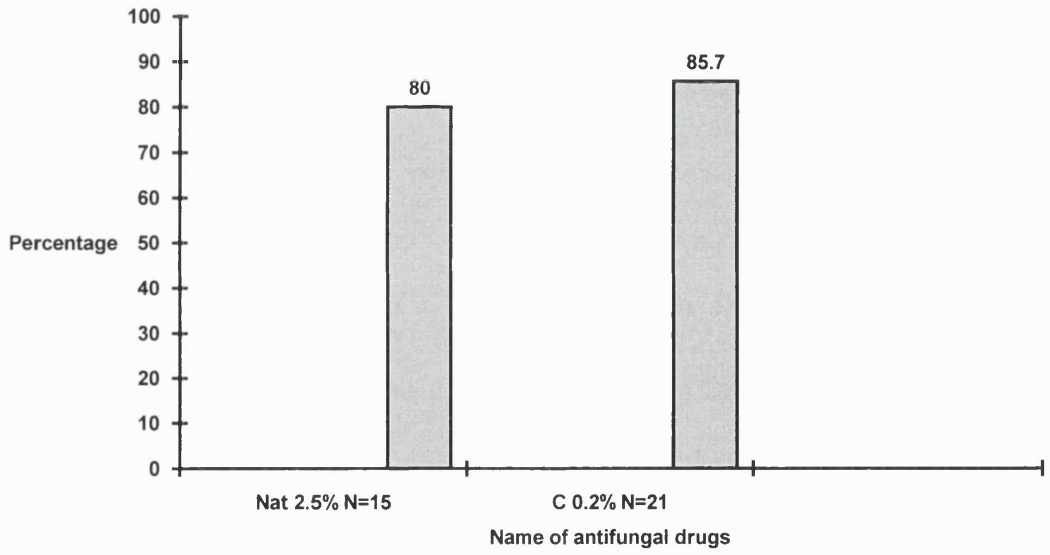
C = chlorhexidine

Nat = natamycin

N = number of patients

Graph-18

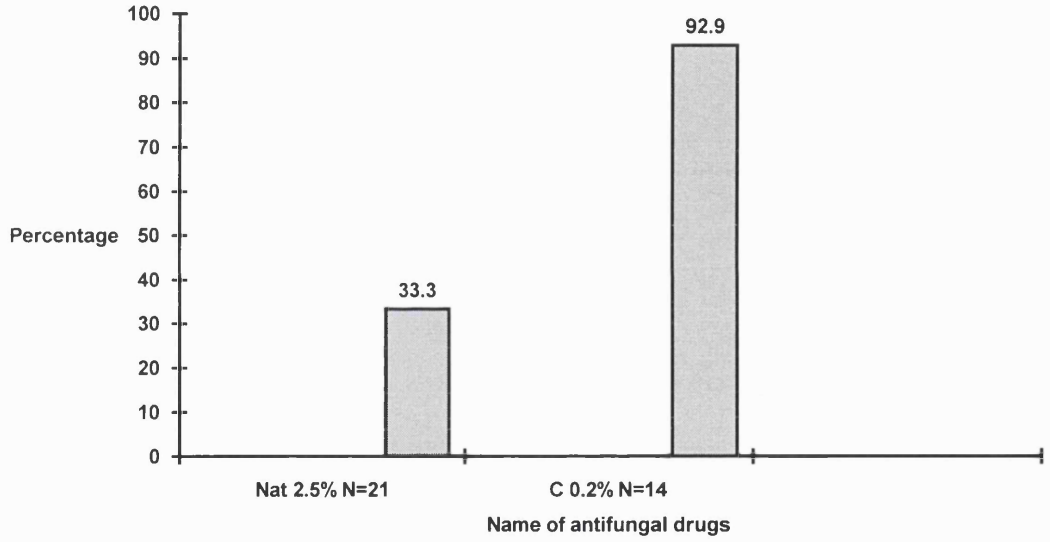
Outcome of favourable response at 5 days in the ulcer size less than 12 sq. mm., in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.



C = chlorhexidine
Nat = natamycin
N = number of patients

Graph-19

Outcome of favourable response at 5 days in the ulcer size more than 12 sq. mm., in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.



C = chlorhexidine
Nat = natamycin
N = number of patients

Table-44

Results at 21 days of antifungal drugs in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Name of drugs	No. of patients	Ulcers healed by 21 days	Drop out	No response	Improving but not healed by 3 week
0.2% chlorhexidine	32	14 (43.75%)	01	14 (48.48%)	03*
2.5% natamycin	33	10 (30.30%)	01	23 (69.69%)	00

*Eventually healed, they were all severe ulcers.

Table-45

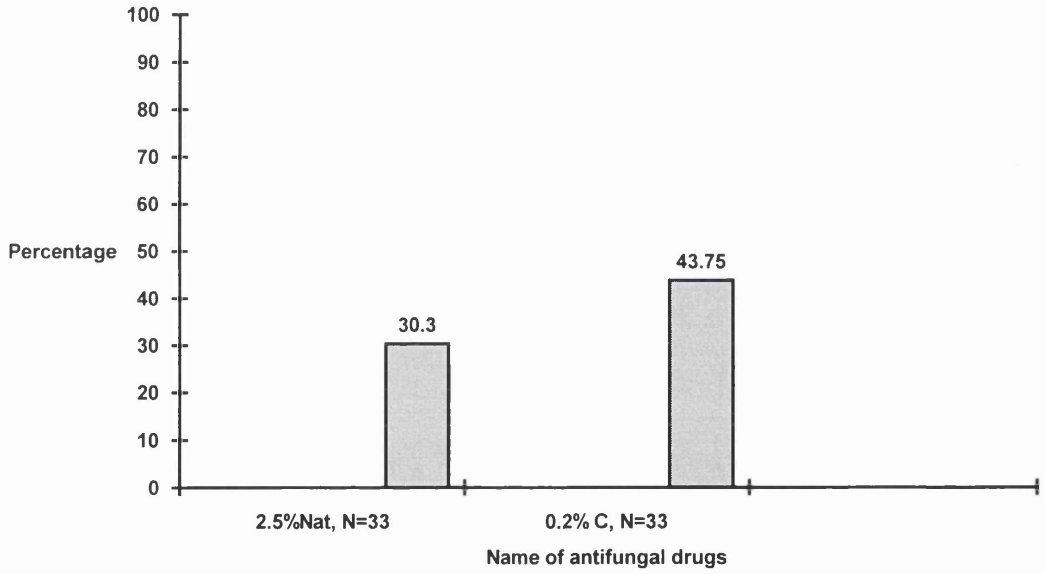
Comparing the efficacy of the 2 treatment groups in producing cure at day 21, with no adjustment for confounding in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Treatment	Cured at day 21	Not cured at day 21	Total no. of patients	% cured		p - value
Chlorhexidine 0.2%	14	18	32	43.75	a**	0.265
Natamycin 2.5%	10	23	33	30.30	b	

**Relative Efficacy (RE = a / b) = 1.44
95% confidence limits for RE 0.75 - 2.76.

Graph-20

Percentage of overall healed fungal keratitis at 21 days in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.



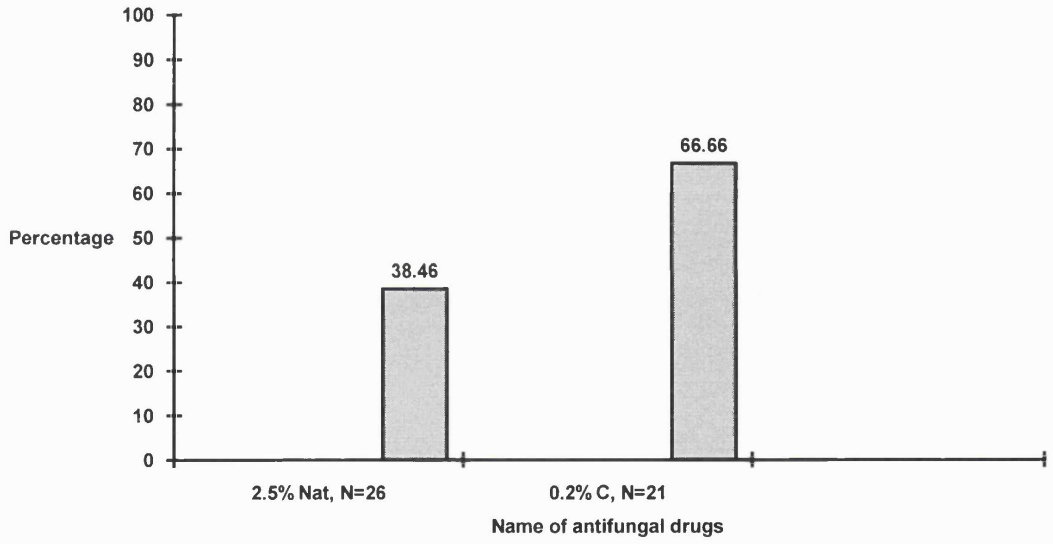
C = chlorhexidine

Nat = natamycin

N = total number of patients

Graph-21

Percentage of healed non-severe fungal keratitis at 21 days in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.



C = chlorhexidine

Nat = natamycin

N = total number of patients

Table-46

Comparing the relative efficacy of the 2 treatment groups in producing cure at day 21, after adjustment for confounding in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Factors adjusted for	Relative Efficacy (RE) of the 2 treatments. (chlorh./natam.)	95% conf. limits for RE	p- value
Age	1.58	0.78 - 3.21	0.193
Sex	1.40	0.69 - 2.83	0.323
Duration of ulcer	1.39	0.72 - 2.69	0.317
Prior antibiotic treatment	1.53	0.77 - 3.04	0.213
Severity of ulcer	1.73	0.98 - 3.07	0.057
Ulcer size	1.16	0.61 - 2.21	0.638
Degree of epithelial defect	1.36	0.62 - 2.96	0.454
Depth of ulcer	1.68	0.92 - 3.08	0.087
Degree of hypopyon	1.29	0.67 - 2.51	0.408
Degree of infiltrate	1.49	0.77 - 2.92	0.224

Table-47

Ultimate fate of ulcers except those healed by 21 days, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Treatment groups	No. of patients	Healed beyond 21 days	Still under alternate treatment	Healed by alternate treatment	Anterior staphyloma	Adherent leucoma	Penetrating keratoplasty	Enucleation	Evisceration
Ulcers not severe									
0.2% chlorhexidine	07	00	00	03	01	02	00	00	01
2.5% natamycin	15	00	00	08	03	02	01	01	00
Severe ulcers									
0.2% chlorhexidine	11	03	01	01	03	02	00	02	00
2.5% natamycin	07	00	00	00	03	03	01	00	00
Total	41	03	01	12	10	09	02	03	01

Table-48

Organisms of the 2 treatment groups in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

	Number of patients		
Organism	Chlorhexidine 0.2%	Natamycin 2.5%	Total
Aspergillus sp.	06	08	14
Aspergillus fumigatus	03	05	08
Coelomycete	00	01	01
Colletotrichum dematium	00	01	01
Curvularia	04	01	05
Cylindrocarpon	02	01	03
Dreschlera	00	01	01
Fusarium sp.	10	12	22
Lasiodiplodia theobromae	02	02	04
Unidentified	03	01	04
None	04	00	04
Not done	01	03	04
Total	35	36	71

Table-49

Eye affected in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Eye	0.2% chlorhexidine	2.5% natamycin	Total number	Percentage
Right eye	20	18	38	53.52%
Left eye	15	18	33	46.47%

Table-50

Sex distribution of patients in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Sex	0.2% chlorhexidine	2.5% natamycin	Total no. of patients	Percentage
Male	25	28	53	74.64%
Female	10	08	18	25.35%

Table-51

Age distribution of patients in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Age (Years)	0.2% chlorhexidine	2.5% natamycin	Total number	Percentage
0-10	00	01	01	1.40%
11-20	02	03	05	7.04%
21-30	10	12	22	30.98%
31-40	08	07	15	21.12%
41-50	08	05	13	18.30%
51-60	04	05	09	12.67%
61-70	02	03	05	7.04%
71-80	01	00	01	1.40%

Table-52

Occupation of patients in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Occupations	0.2% chlorhexidine	2.5% natamycin	No. of patients	Percentage
Farmer	12	13	25	35.21%
Labourer	03	05	08	11.26%
Businessman	02	02	04	5.63%
Student	02	01	03	4.22%
House wife	09	08	17	23.94%
Service	02	03	05	7.04%
Mechanic	01	03	04	5.63%
Driver	01	00	01	1.40%
Carpenter	01	00	01	1.40%
Rickshaw puller	02	01	03	4.22%

Table-53

Predisposing factors of fungal keratitis in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Predisposing factors		0.2% chlorhexidine	2.5% natamycin	Total no. of patients	Percentage
Trauma		22	18	40	56.33%
	Foreign body (Unknown)	03	02	05	7.04%
	Straw	01	00	01	1.40%
	Finger nail	01	00	01	1.40%
	Bamboo	00	01	01	1.40%
	Heat	01	00	01	1.40%
	Stone	01	00	01	1.40%
	Soil	00	01	01	1.40%
	Thorn	00	01	01	1.40%
	Wood	01	01	02	2.81%
	Paddy grain	08	05	13	18.30%
	Insect	02	04	06	8.45%
	Sand	02	02	04	5.63%
No history of trauma		13	18	31	43.66%

Table-54

Medications prior to corneal scraping in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Name of drugs	0.2% chlorhexidine	2.5% natamycin	No. of patients	Percentage
Antibiotic	20	17	37	52.11%
Steroids	00	02	02	2.81%
Antifungal	00	01	01	1.40%
Snail juice	01	00	01	1.40%
Homeopathic eye drops	00	01	01	1.40%
Eye drops (Unknown)	10	10	20	28.16%
Antibiotic & antifungal	01	03	04	5.63%
Steroid & antiviral	00	01	01	1.40%
Antibiotic & steroid	00	01	01	1.40%
Antibiotic & antiviral	01	00	01	1.40%
Antibiotic, antifungal & steroid	01	00	01	1.40%
None	01	00	01	1.40%

Table-55

Duration of illness prior to corneal scraping in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Duration (Days)	0.2% chlorhexidine	2.5% natamycin	No. of patients	Percentage
1 to 7	09	06	15	21.12%
8 to 14	12	13	25	35.21%
15 to 21	08	11	19	26.76%
22 to 30	05	06	11	15.49%
31 to 60	01	00	01	1.40%

Table-56

Visual acuity of patients prior to corneal scraping in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Visual acuity	0.2% chlorhexidine	2.5% natamycin	Total no. of patients	Percentage
6/6	01	00	01	1.40%
6/9	02	01	03	4.22%
6/18	00	01	01	1.40%
6/24	04	01	05	7.04%%
6/36	04	05	09	12.67%
6/60	09	04	13	18.30%
C.F.	05	07	12	16.90%
H.M.	08	15	23	32.39%
P.L.	02	02	04	5.63%

Table-57

Visual acuity of patients after healing of corneal ulcers treated with 0.2% chlorhexidine in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Study number	Total no. of patients	Initial vision	Final vision
Ulcers not severe			
02		6/60	6/24
04		6/24	6/18
10		6/60	6/36
13		6/9	6/6
33		6/60	6/18
36		6/36	6/18
38		H.M.	6/18
46		6/24	6/18
49		6/60	6/18
50		6/6	6/6
55		6/60	6/24
60		6/36	6/18
61		6/60	6/36
66		C.F-3 feet	6/18
	14		
Severe ulcers			
08		6/60	6/12
25		C.F-3 feet	3/60
44		H.M.	6/12
	03		

Table-58

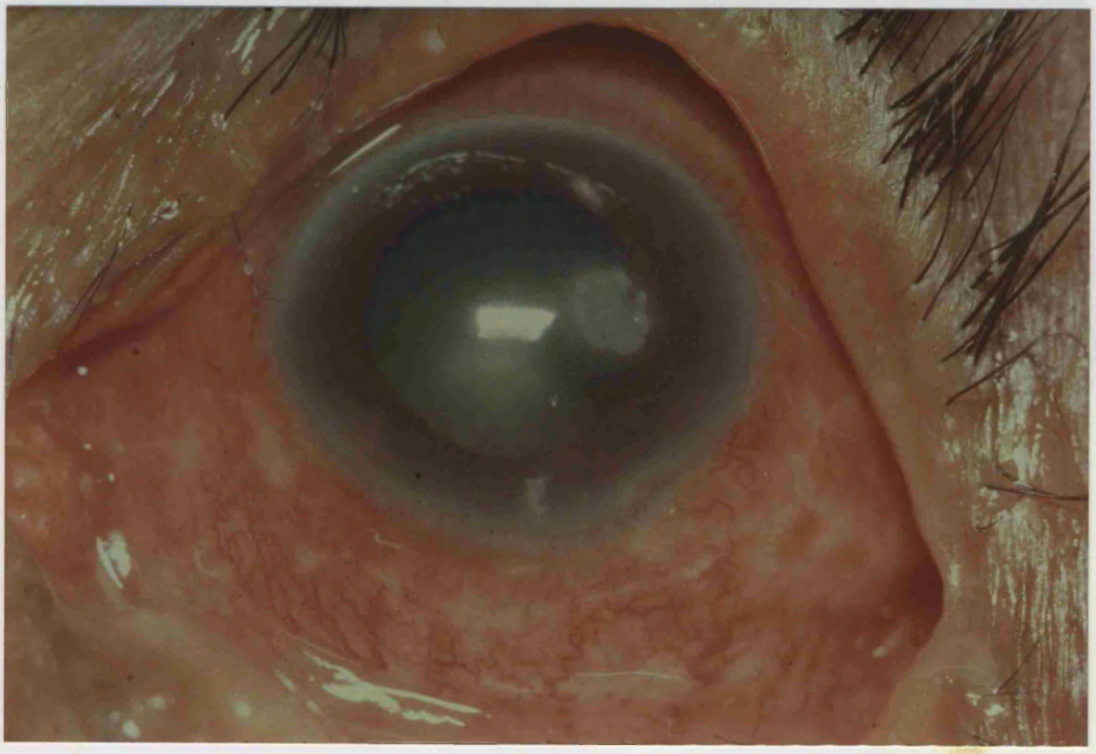
Visual acuity of patients after healing of corneal ulcers treated with 2.5% natamycin in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Study no.	Total no. of patients	Initial vision	Final vision
Ulcers not severe			
03		6/24	6/9
18		H.M.	6/6
22		6/60	6/9
31		C.F-1 foot	6/60
39		6/36	6/24
41		H.M.	3/60
42		C.F-1 foot	6/60
51		6/60	6/18
56		H.M.	C.F-1 foot
64		6/36	6/9
	10		

Illustration - 2

Showing gradual improvement of fungal corneal ulcer of patient no. 2 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

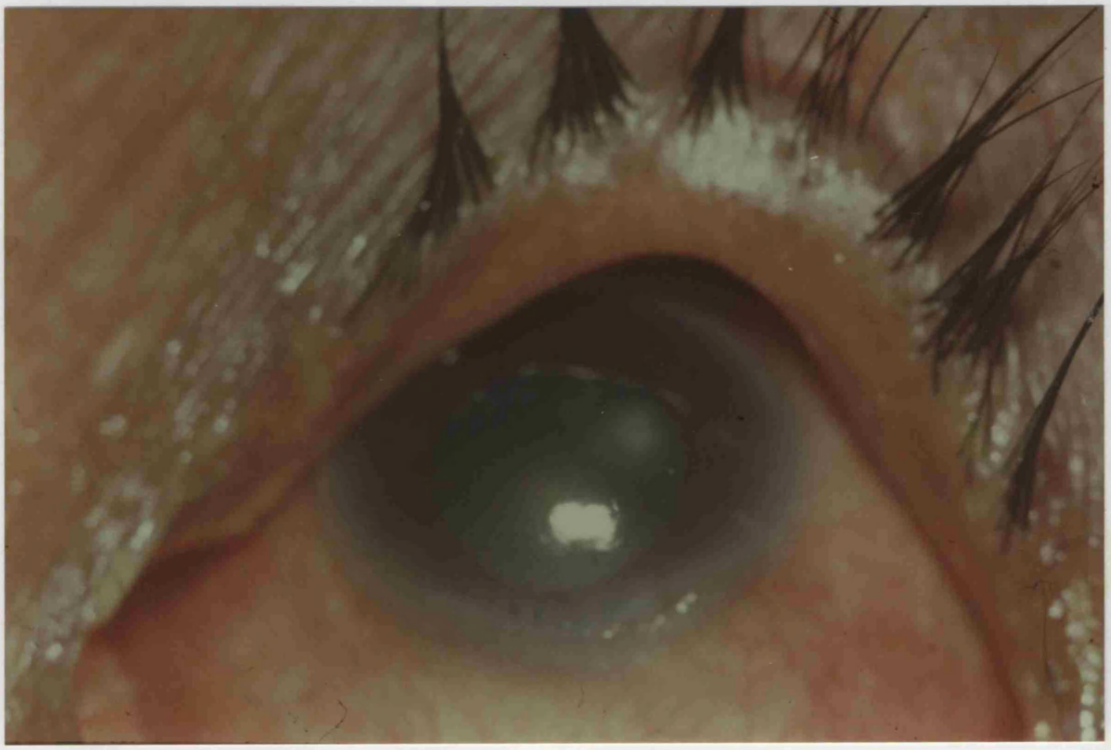
Name-Pushpa Bala Mullick.
Age-70 years.
Sex-Female.
Occupation-House wife.
Predisposing factor-Trauma by straw.
Nature of injury-Nonsevere.
Fungus identified-Cylindrocarpon sp.



Ulcer at presentation

Illustration - 2

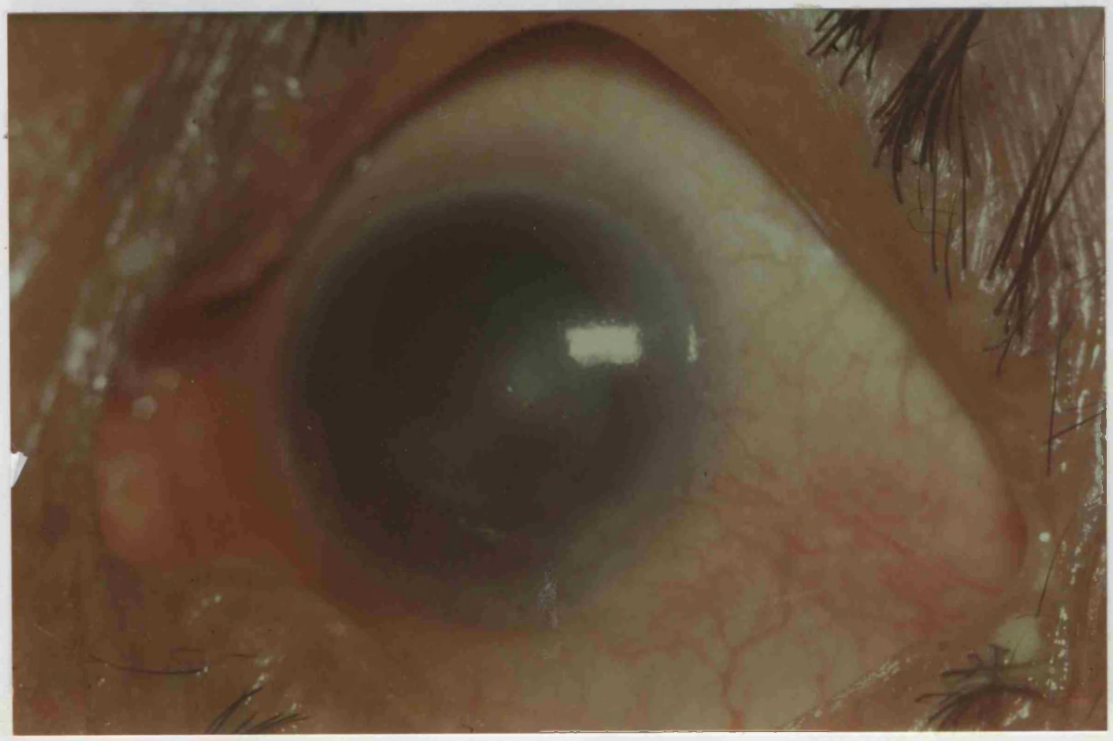
Showing gradual improvement of fungal corneal ulcer of patient no. 2 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Ulcer on the 5th day

Illustration - 2

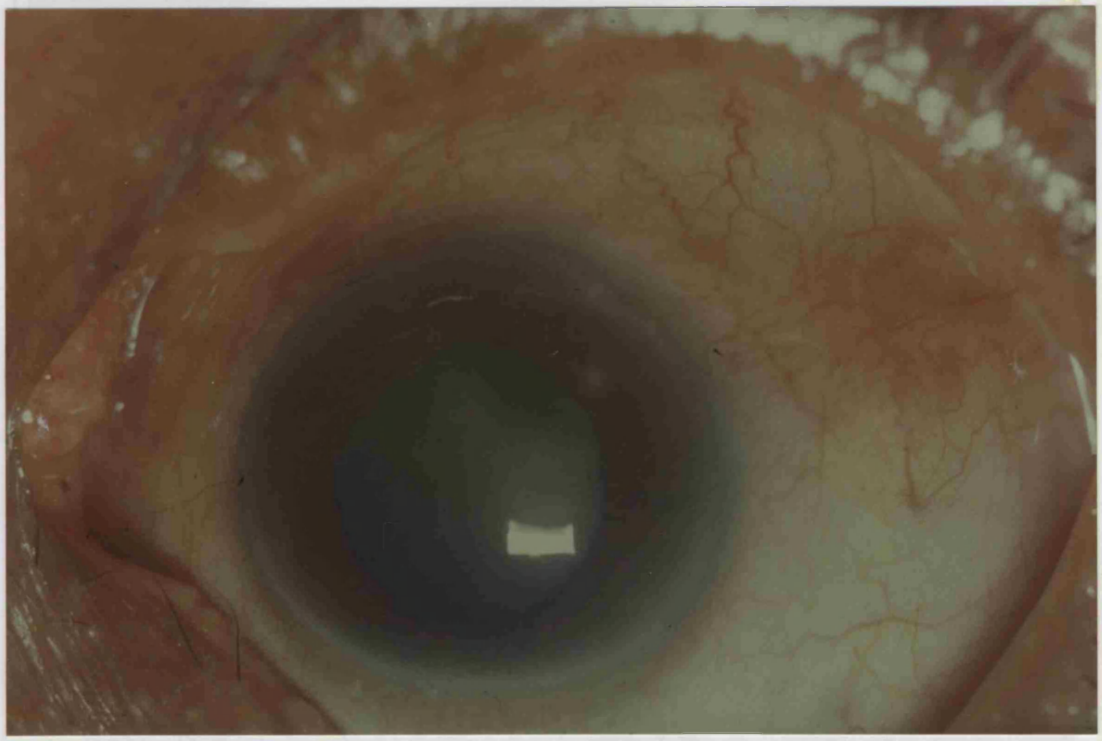
Showing gradual improvement of fungal corneal ulcer of patient no. 2 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Ulcer on the 7th day

Illustration - 2

Showing gradual improvement of fungal corneal ulcer of patient no. 2 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

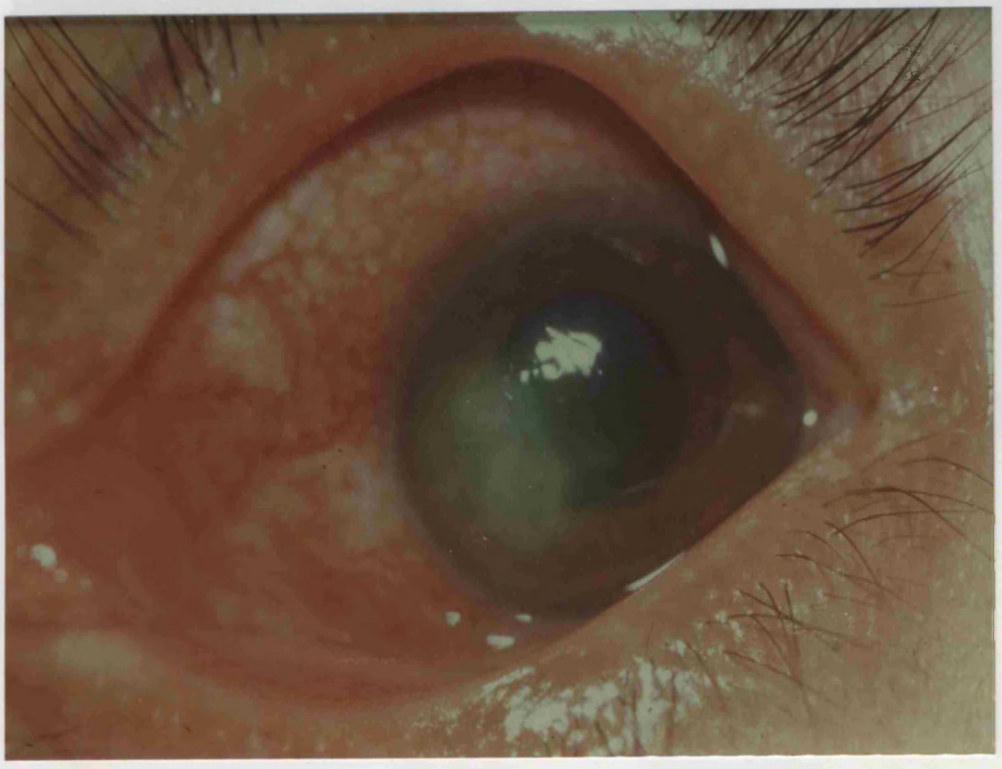


Ulcer healed on the 21st day

Illustration - 3

Showing gradual improvement of fungal corneal ulcer of patient no. 4 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

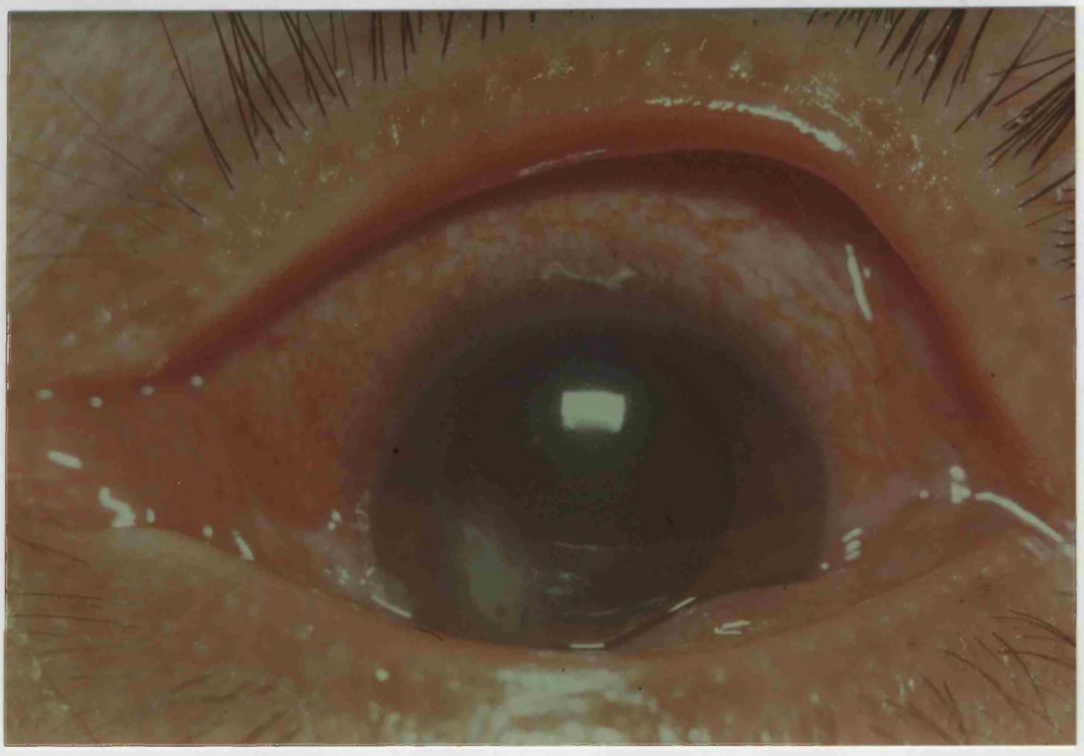
Name-Zibana Bala.
Age-45 years.
Sex-Female.
Occupation-Farmer.
Predisposing factor-No history of trauma.
Nature of injury-Nonsevere.
Fungus identified-Fusarium sp.



Ulcer at presentation

Illustration - 3

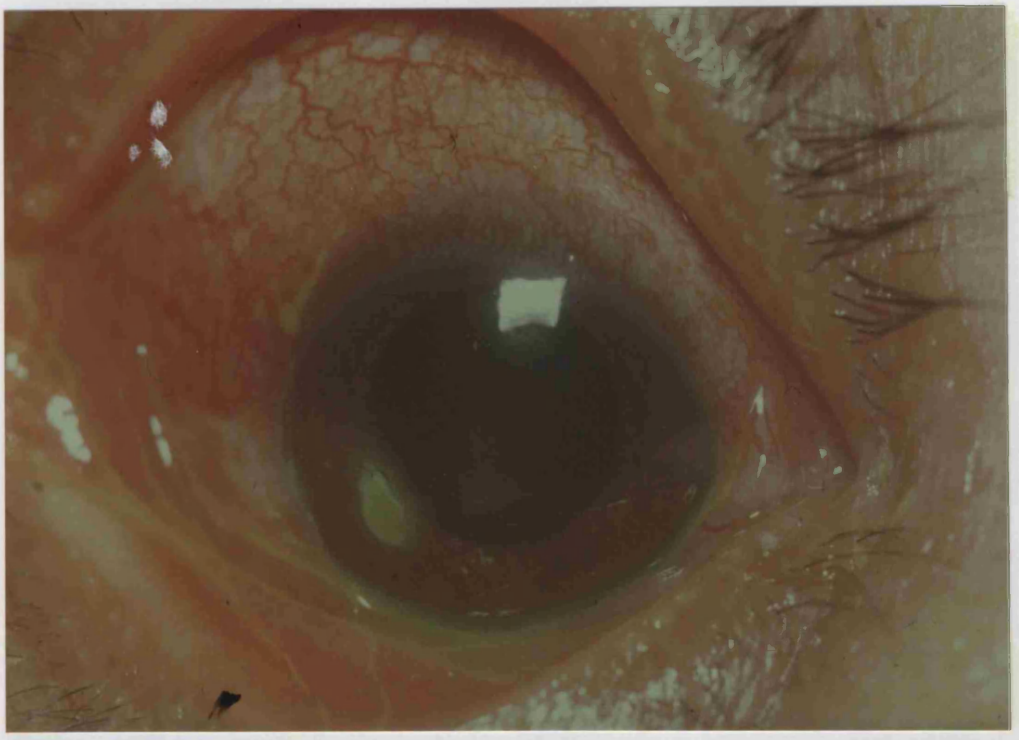
Showing gradual improvement of fungal corneal ulcer of patient no. 4 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Ulcer on the 5th day

Illustration - 3

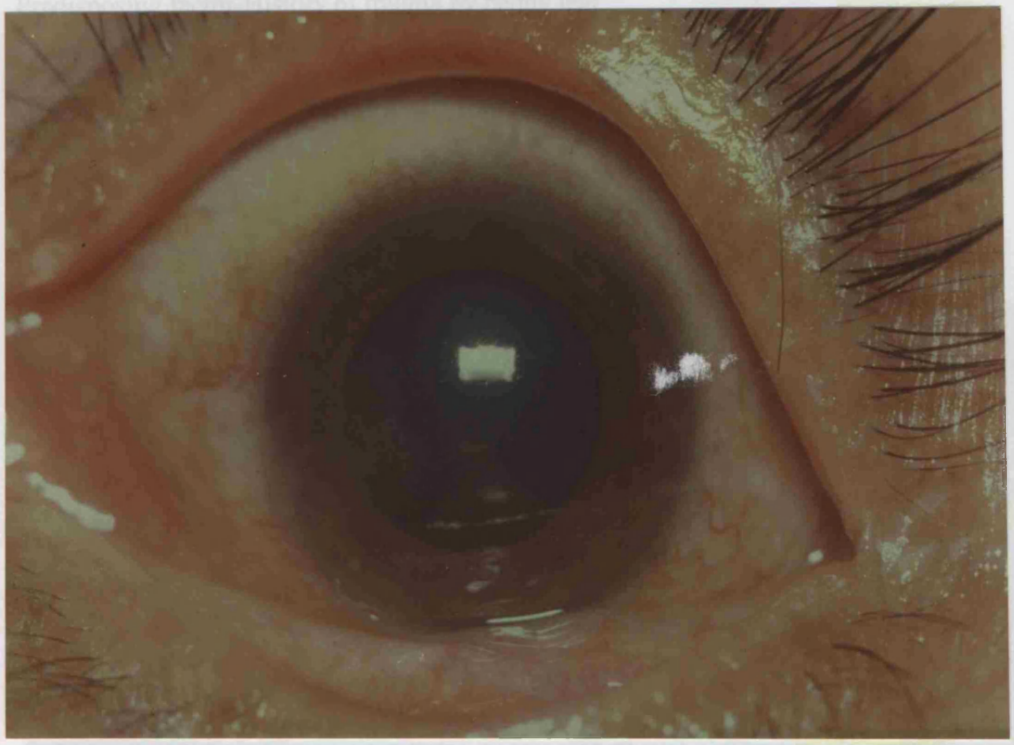
Showing gradual improvement of fungal corneal ulcer of patient no. 4 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Ulcer on the 7th day

Illustration - 3

Showing gradual improvement of fungal corneal ulcer of patient no. 4 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

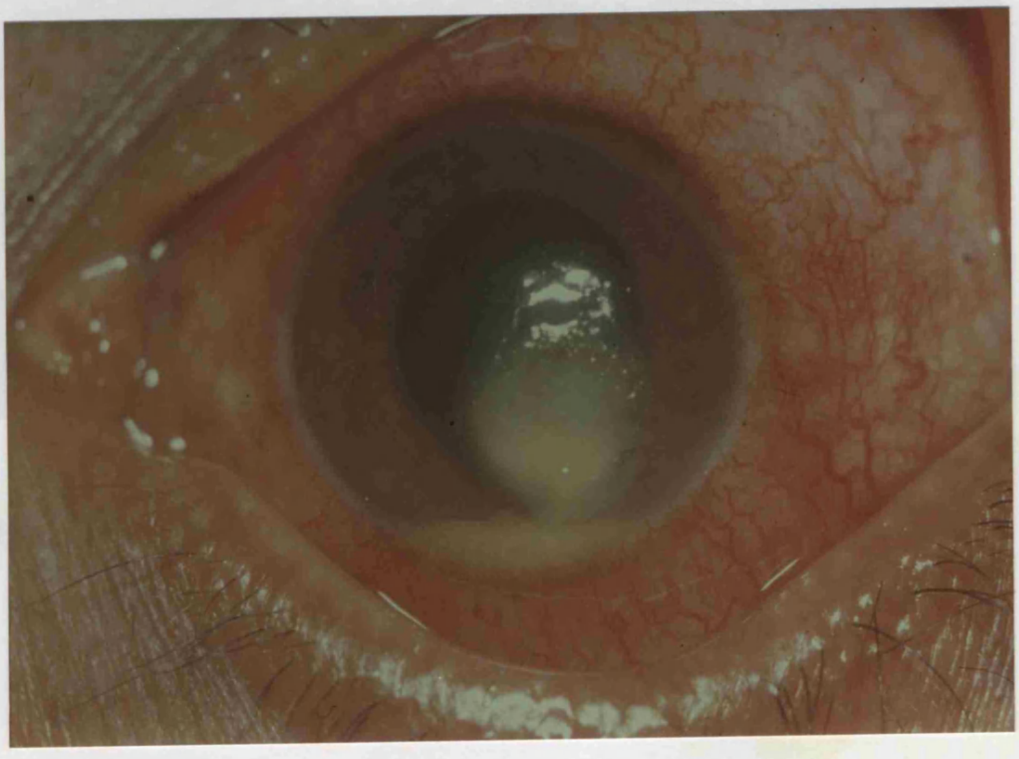


Ulcer healed on the 17th day

Illustration - 4

Showing gradual improvement of hypopyon of patient no. 8 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

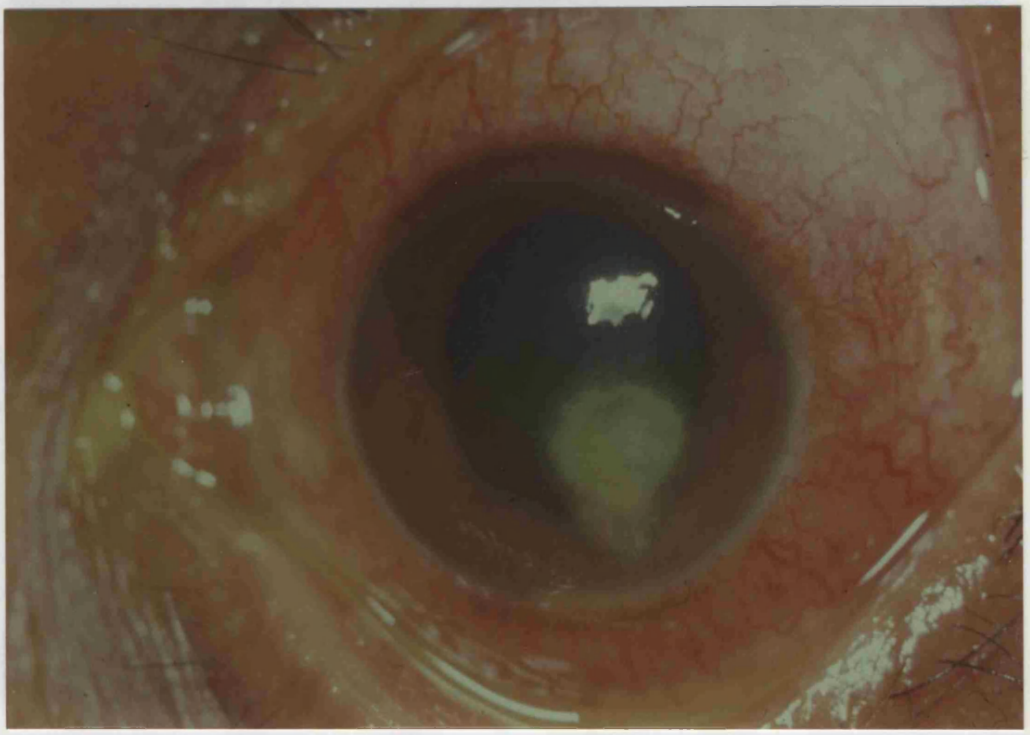
Name-Amir Hussain.
Age-42 years.
Sex-Male.
Occupation-Rickshaw puller.
Predisposing factor-History of trauma by paddy leaf.
Nature of injury-Severe.
Fungus identified-Curvularia sp.



Hypopyon at presentation

Illustration - 4

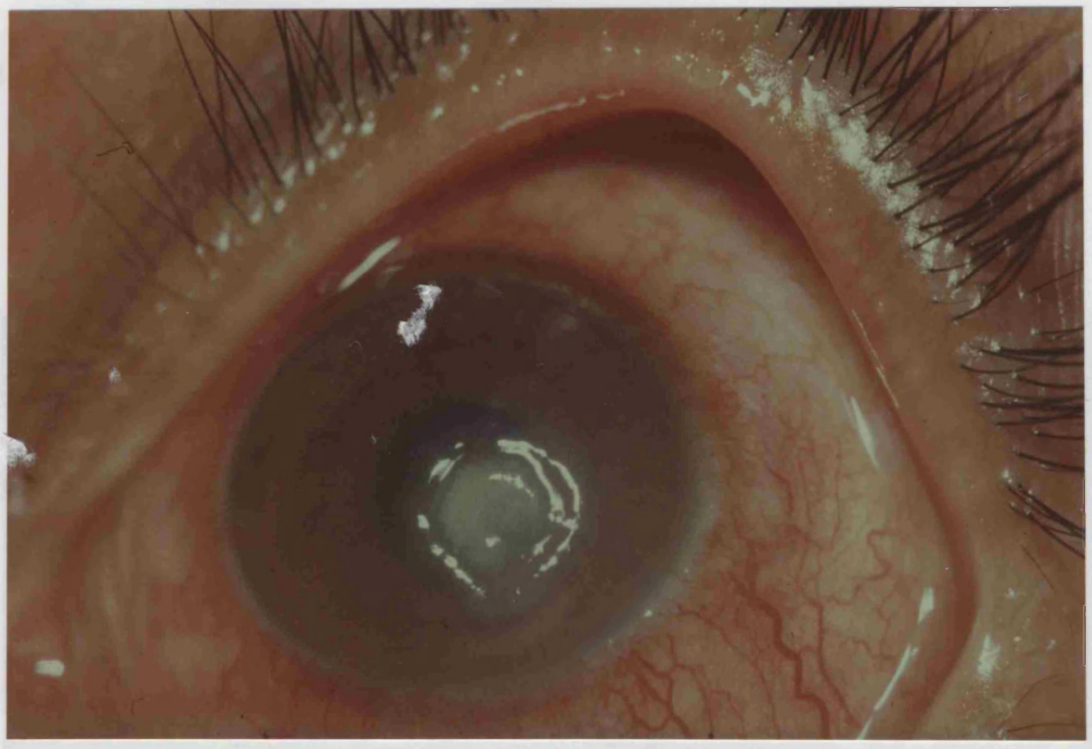
Showing gradual improvement of hypopyon of patient no. 8 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Hypopyon at the 5th day

Illustration - 4

Showing gradual improvement of hypopyon of patient no. 8 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

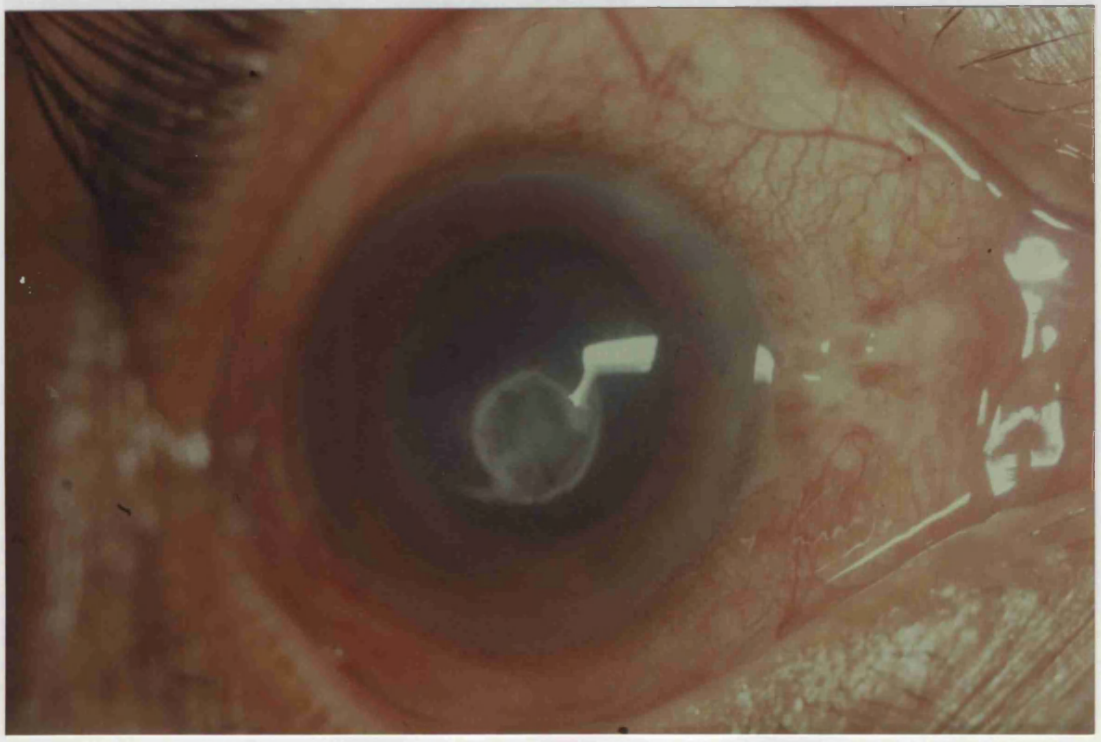


No hypopyon at the 21st day, eventually healed on the 60th day.

Illustration - 5

Showing gradual improvement of fungal corneal ulcer of patient no. 10 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

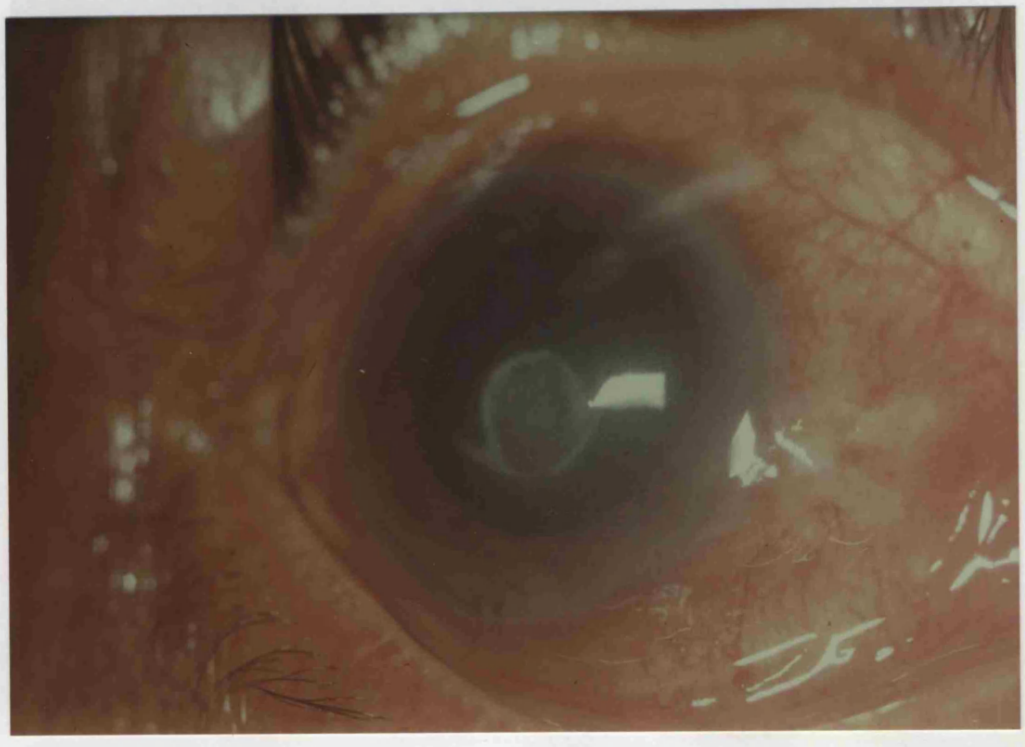
Name-Kamrul Huq.
Age-50 years.
Sex-Male.
Occupation-Farmer.
Predisposing factor-No history of trauma.
Nature of injury-Non-severe.
Fungus identified-No growth.



Ulcer at presentation

Illustration - 5

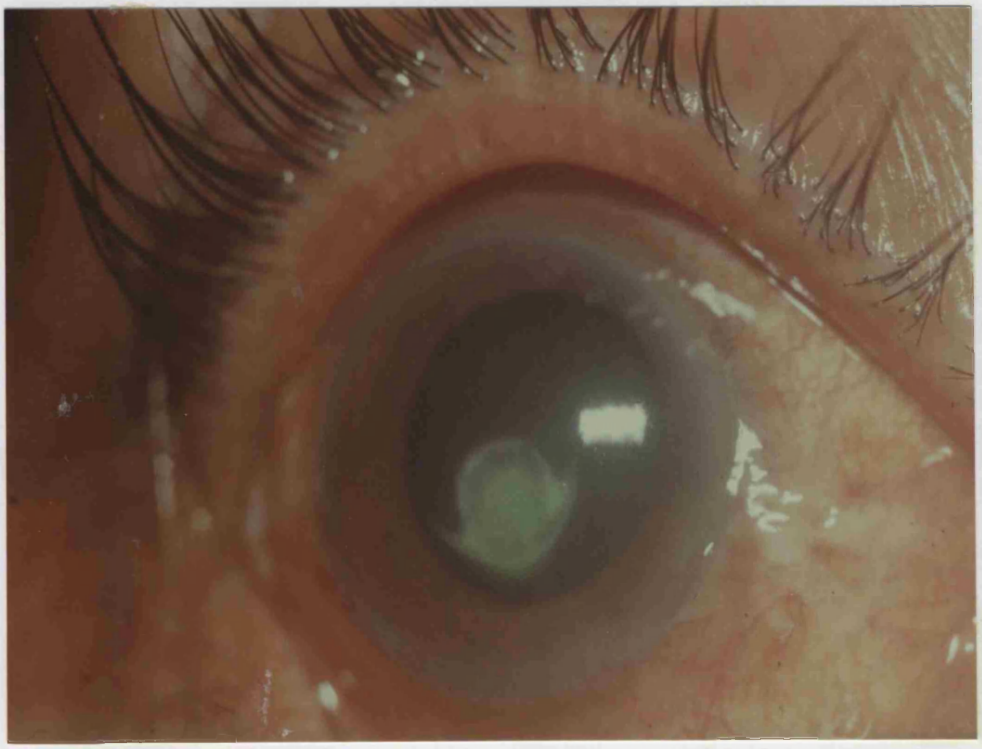
Showing gradual improvement of fungal corneal ulcer of patient no. 10 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Ulcer on the 5th day

Illustration - 5

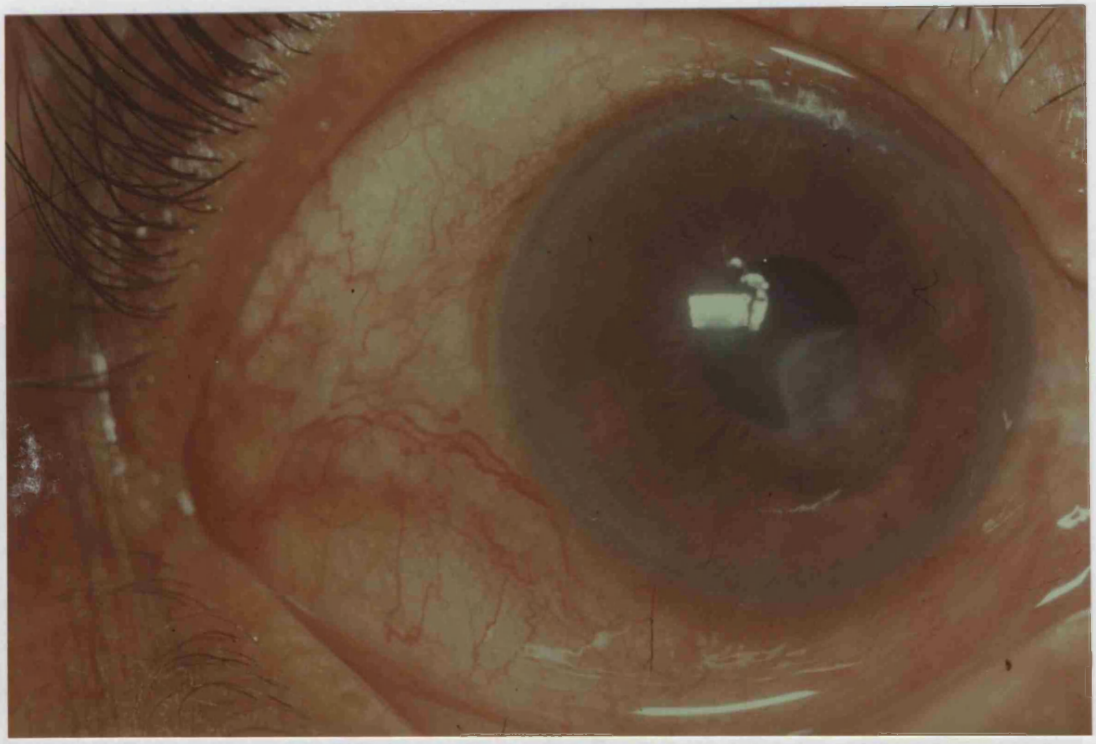
Showing gradual improvement of fungal corneal ulcer of patient no. 10 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Ulcer on the 7th day

Illustration - 5

Showing gradual improvement of fungal corneal ulcer of patient no. 10 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

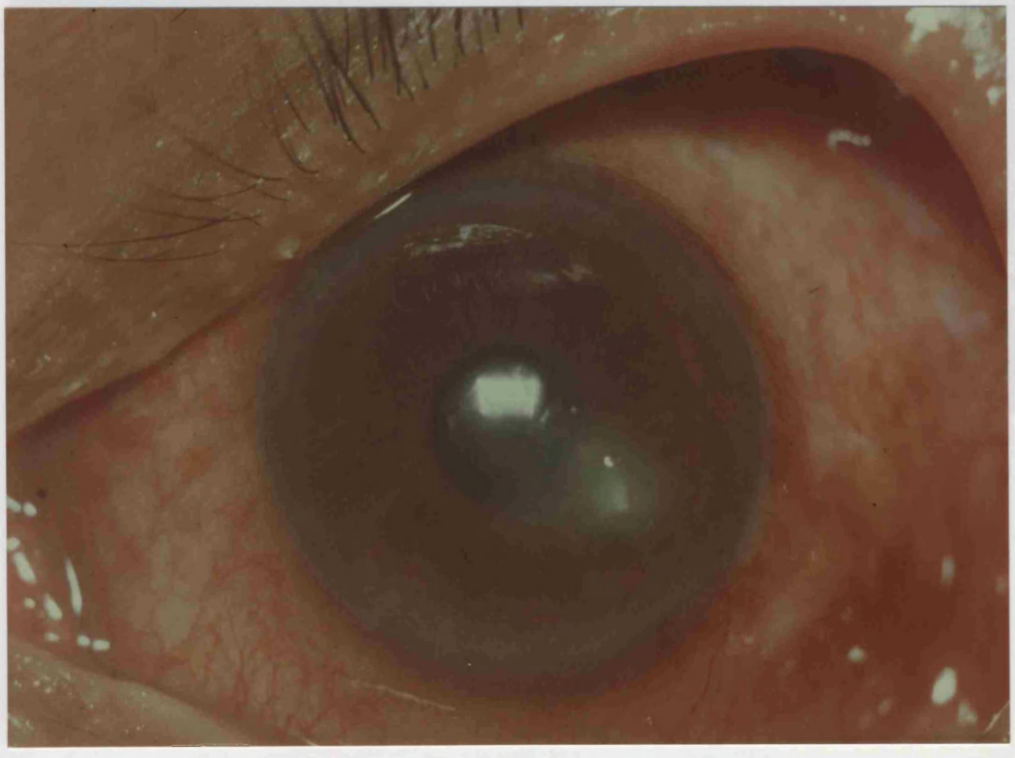


Ulcer healed on the 21st day

Illustration - 6

Showing gradual improvement of fungal corneal ulcer of patient no. 13 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

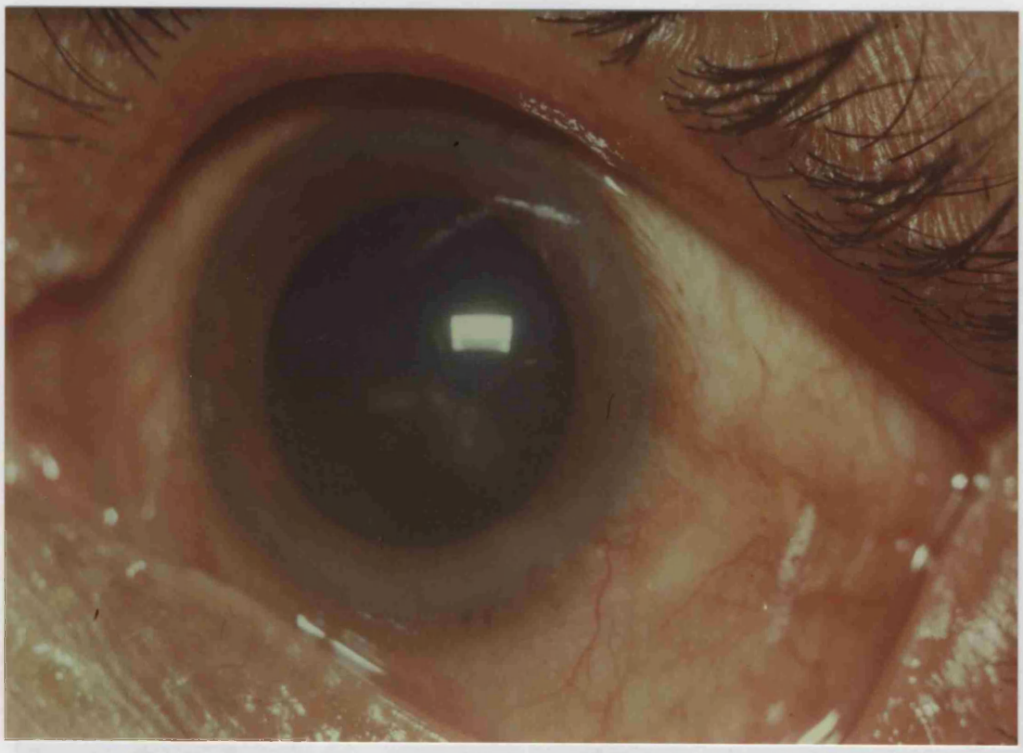
Name-Nagendra Kumar Devnath.
Age-50 years.
Sex-Male.
Occupation-Labourer.
Predisposing factor-Trauma by paddy grain.
Nature of injury-Non-severe.
Fungus identified-No growth.



Ulcer at presentation

Illustration - 6

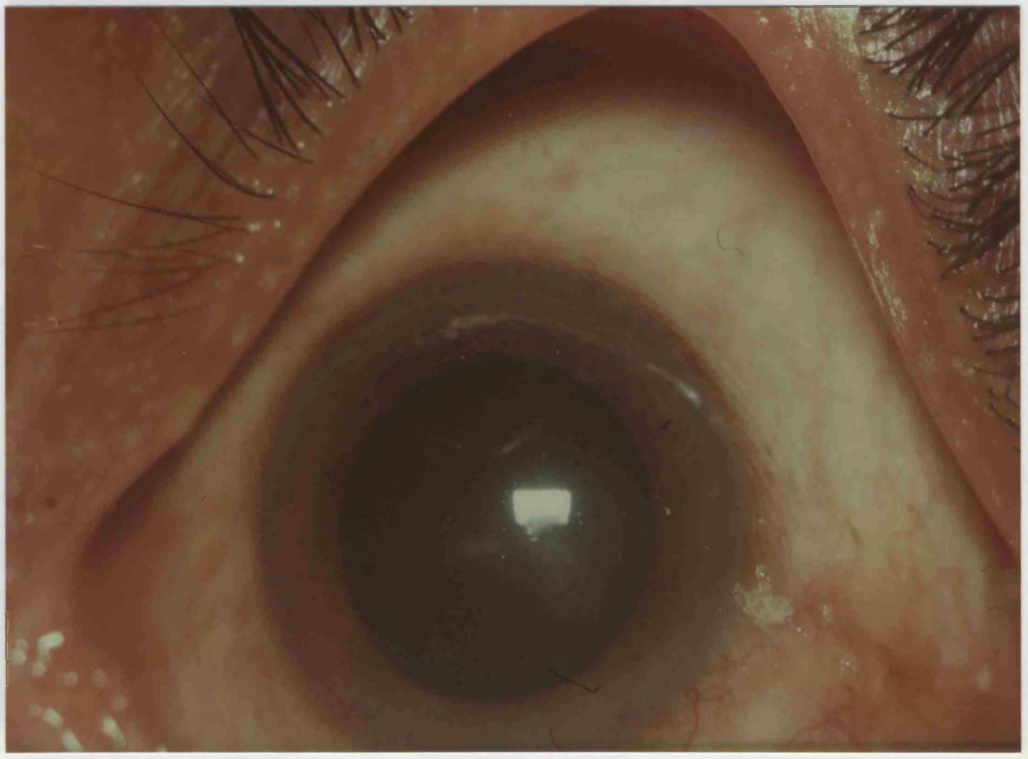
Showing gradual improvement of fungal corneal ulcer of patient no. 13 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Ulcer on the 5th day

Illustration - 6

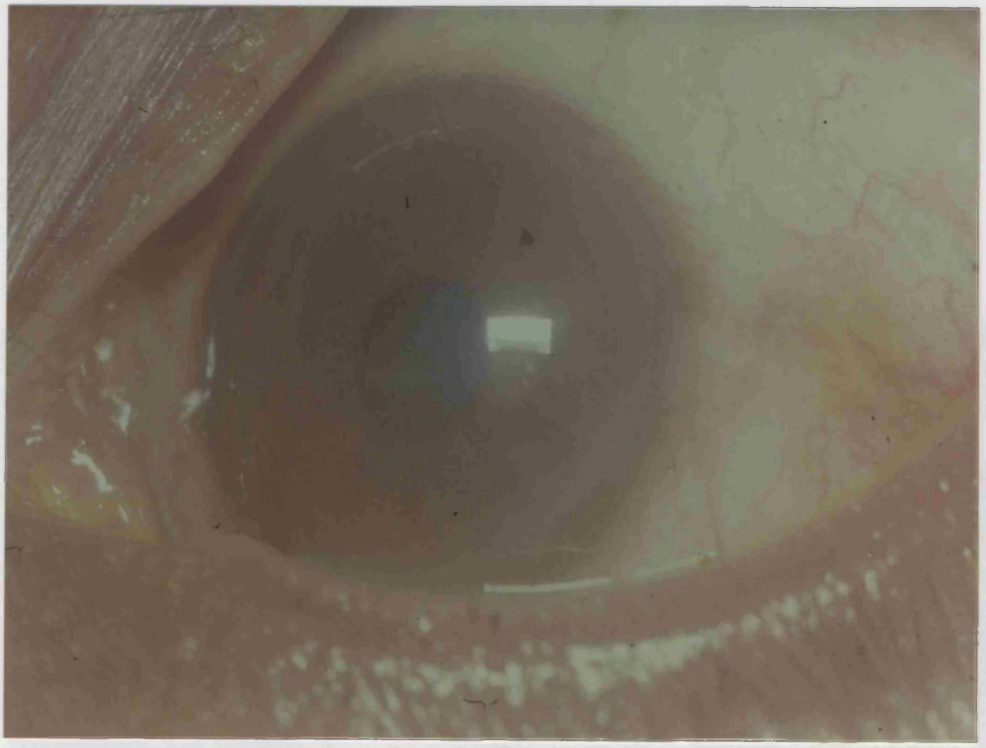
Showing gradual improvement of fungal corneal ulcer of patient no. 13 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Ulcer healed on the 7th day

Illustration - 6

Showing gradual improvement of fungal corneal ulcer of patient no. 13 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

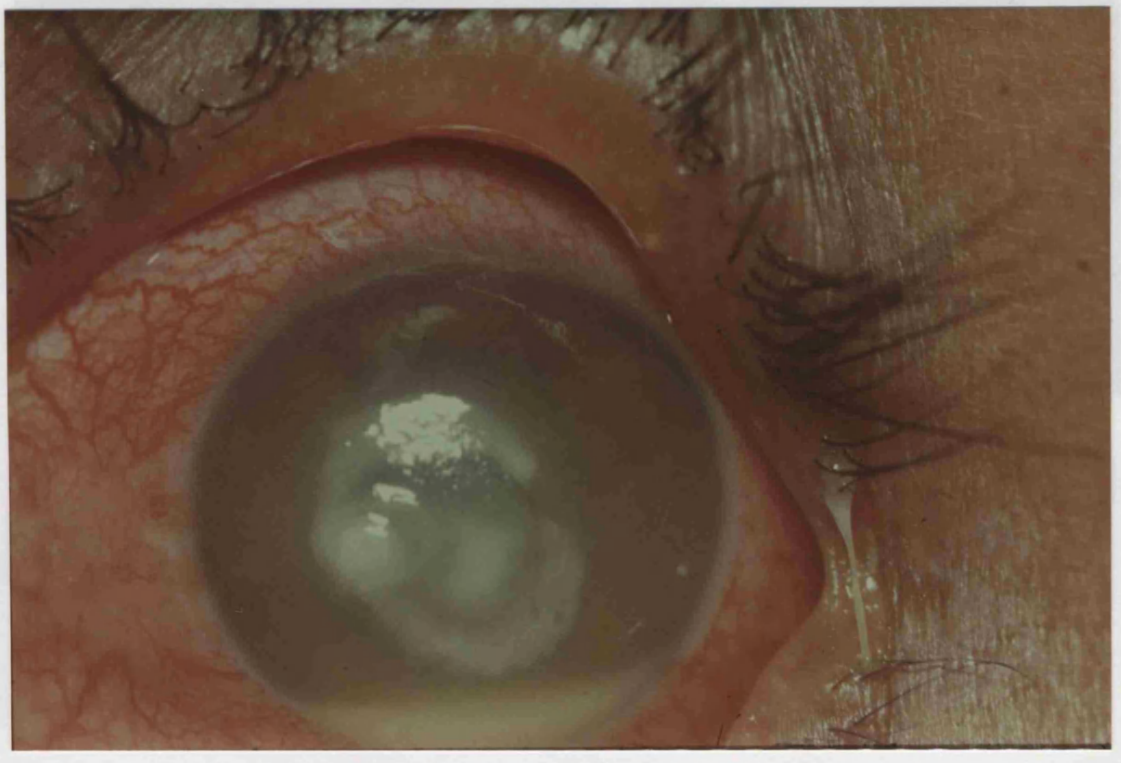


Scar on the 21st day

Illustration - 7

Showing initial improvement, but then deterioration of hypopyon of patient no. 16 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

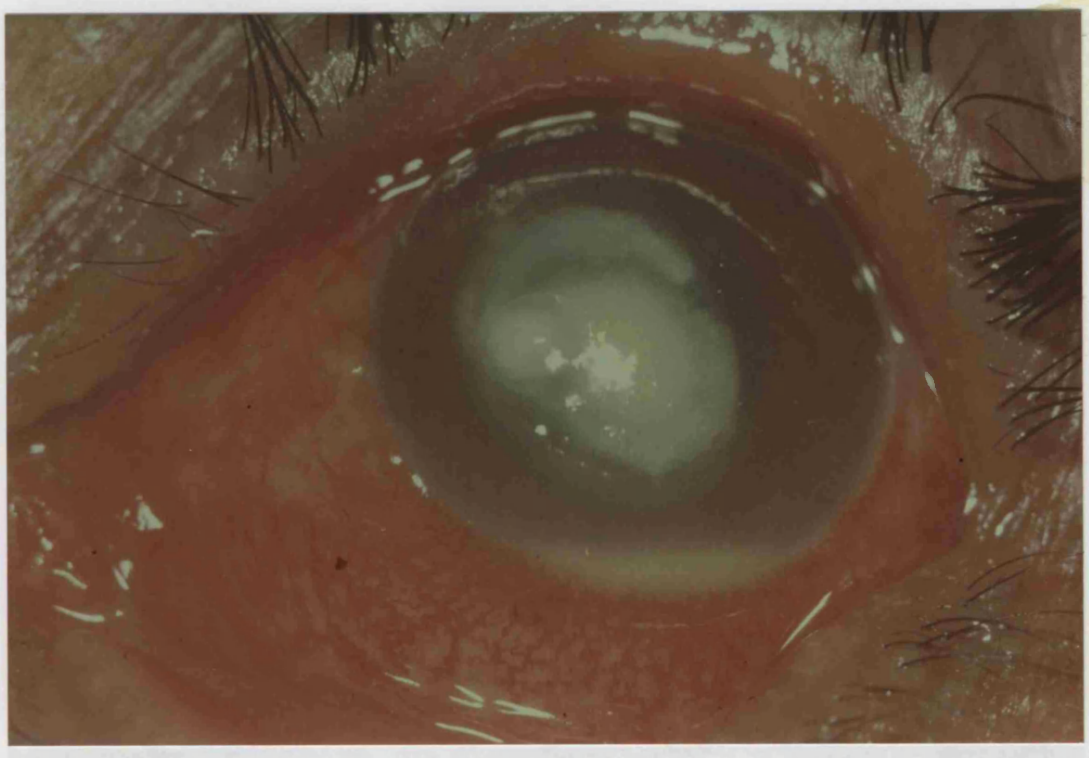
Name-Hafizur Rahman.
Age-30 years.
Sex-Male.
Occupation-Farmer.
Predisposing factor-Trauma by paddy grain.
Nature of injury-Severe.
Fungus identified-Aspergillus fumigatus.



Hypopyon at presentation

Illustration - 7

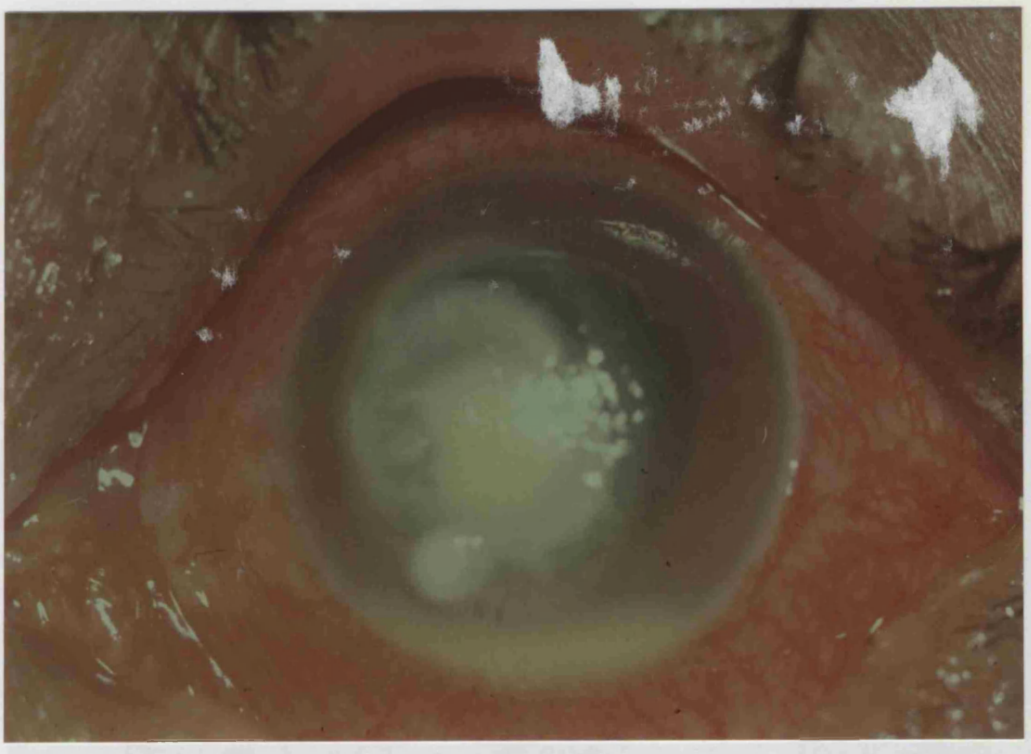
Initial improvement, but then deterioration of hypopyon of patient no. 16 treated by 0.2% chlorhexidine in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Hypopyon on the 5th day

Illustration - 7

Initial improvement, but then deterioration of hypopyon of patient no. 16 treated by 0.2% chlorhexidine in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

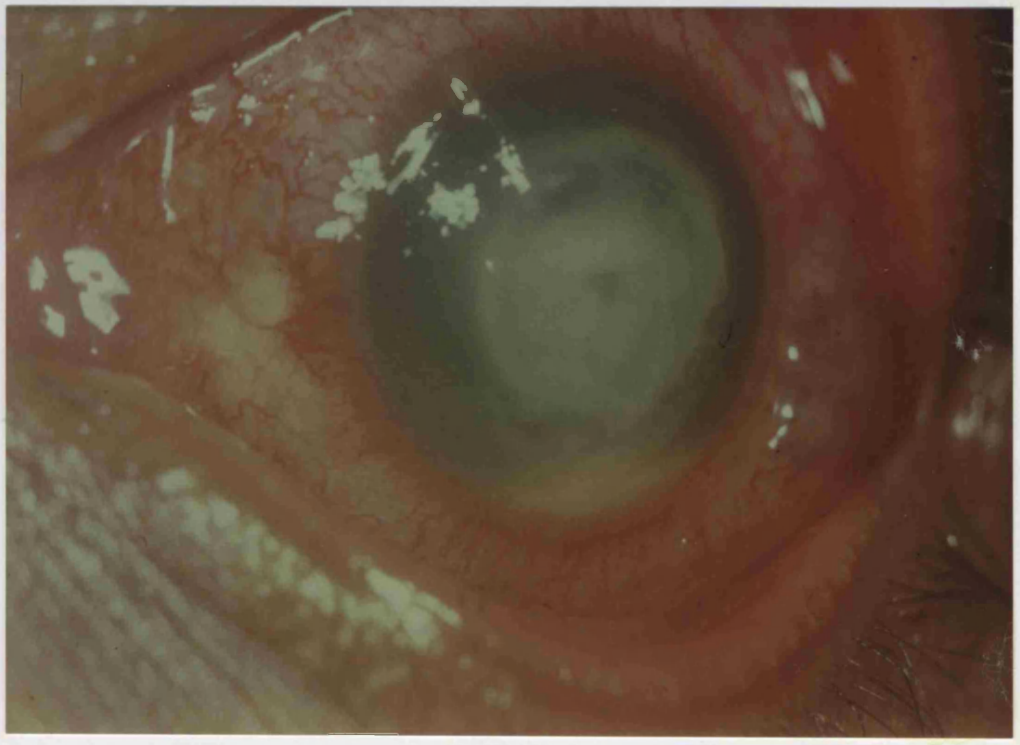


Hypopyon on the 7th day (worse)

Illustration - 8

Showing initial improvement of hypopyon but deterioration of ulcer of patient no. 21 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0 2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

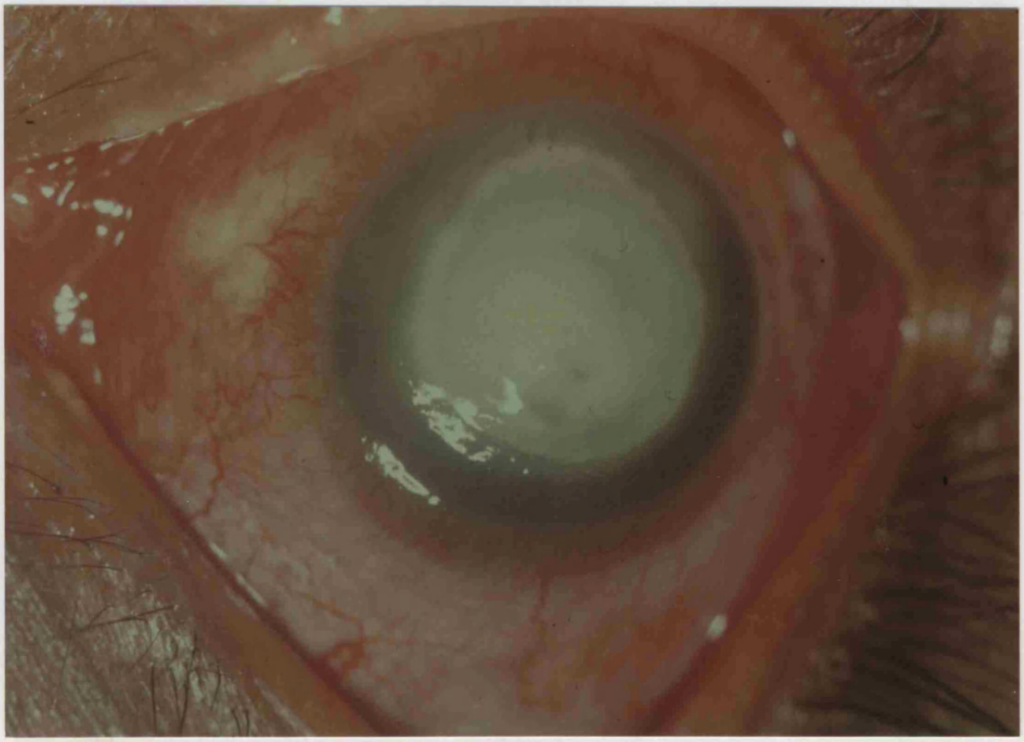
Name-Joynul Abedin.
Age-25 years.
Sex-Male.
Occupation-Farmer.
Predisposing factor-Trauma by paddy grain.
Nature of injury-Severe.
Fungus identified-Aspergillus fumigatus.



Ulcer and hypopyon at presentation

Illustration - 8

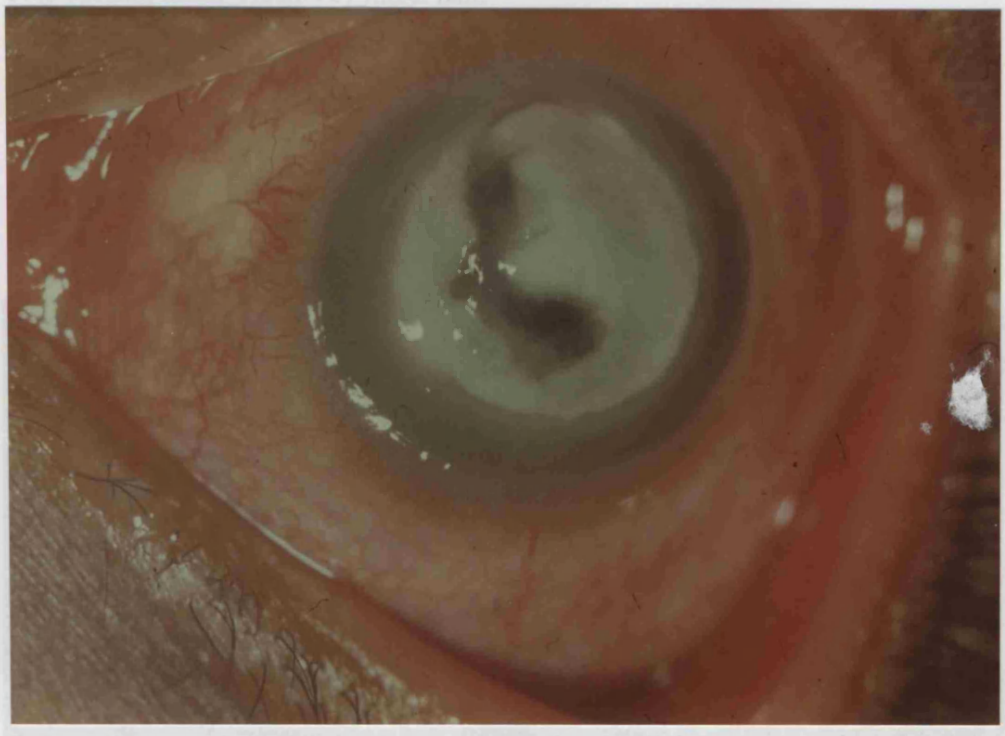
Initial improvement of hypopyon but deterioration of ulcer of patient no. 21 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



No hypopyon on the 5th day

Illustration - 8

Initial improvement of hypopyon but deterioration of ulcer of patient no. 21 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

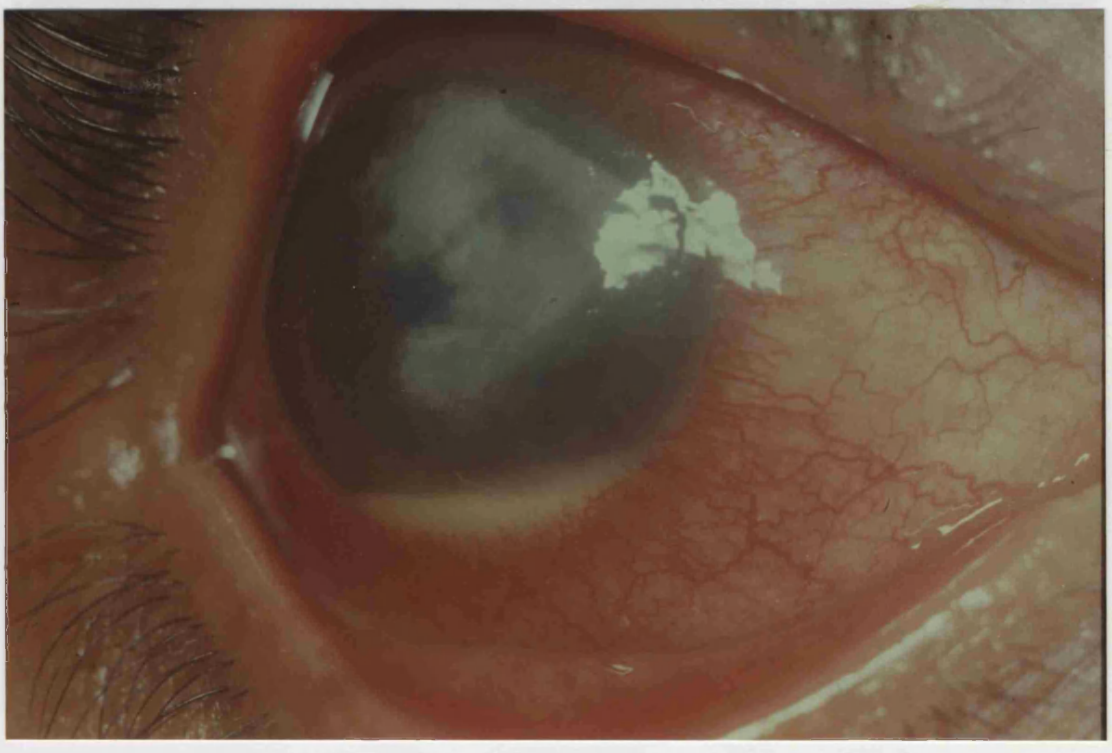


Ulcer on the 7th day (worse)

Illustration - 9

Showing gradual improvement of hypopyon of patient no. 25 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.

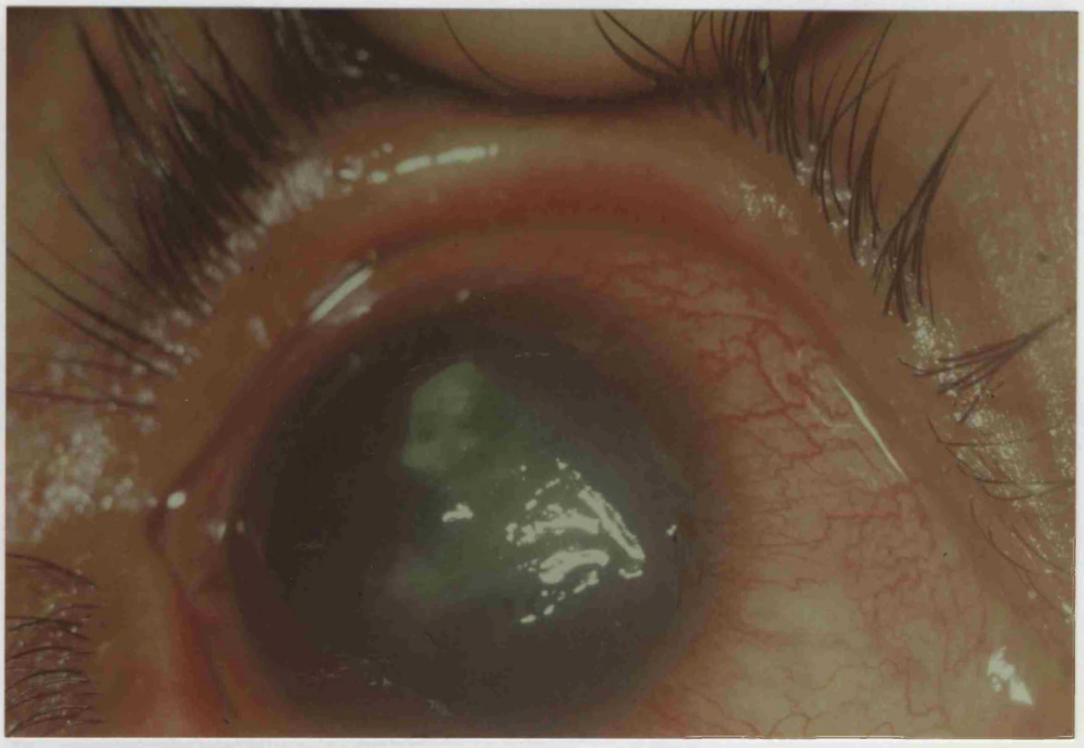
Name-Salim Uddin.
Age-15 years.
Sex-Male.
Occupation-Student.
Predisposing factor-Trauma by finger nail.
Nature of injury-Severe.
Fungus identified-Fusarium sp.



Hypopyon at presentation

Illustration - 9

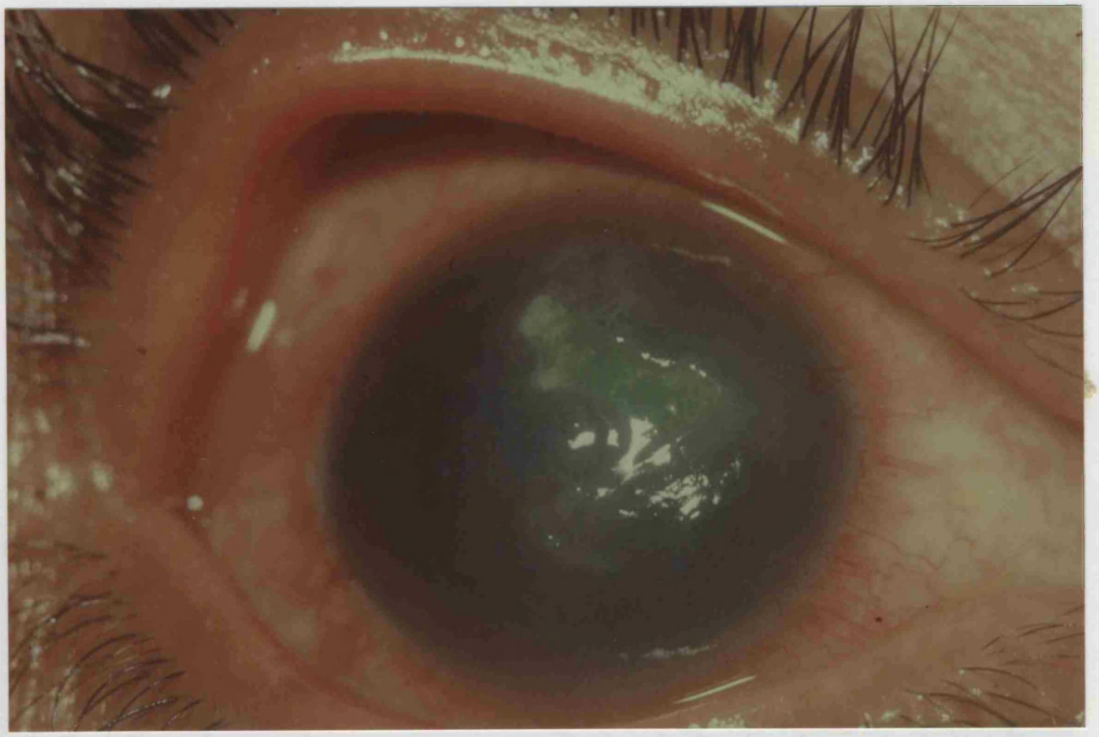
Showing gradual improvement of hypopyon of patient no. 25 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



Ulcer on the 5th day

Illustration - 9

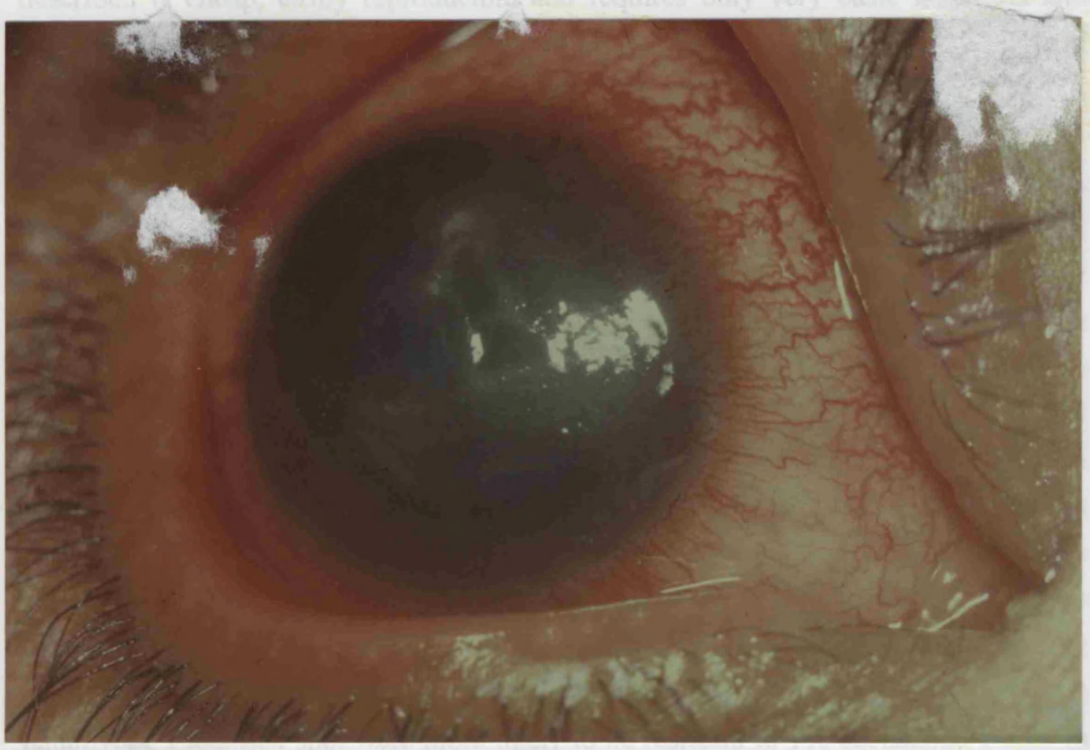
Showing gradual improvement of hypopyon of patient no. 25 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



No hypopyon on the 7th day

Illustration - 9

Showing gradual improvement of hypopyon of patient no. 25 treated by 0.2% chlorhexidine, in the randomised trial of chlorhexidine 0.2% and natamycin 2.5% done in the EITC, Chittagong, Bangladesh.



No hypopyon and ulcer on the 10th day, eventually healed on the 26th day.

6.0. DISCUSSION

6.1. In vitro sensitivities

It is realised that fungal sensitivity testing is a contentious area and that clinical outcome is often independent of sensitivity test results. The objective was to set up a simple method of testing a wide range of fungi against a number of antiseptic and antifungal agents to give a broad indication of their potential efficacy. The method described is cheap, easily reproducible and requires only very basic materials and technical skills. For this reason it can be transported world-wide and used in tropical areas and rural hospitals where these infections are commonly seen.

Identification beyond genus level, while being of academic interest is time consuming, difficult and can be expensive. Once a filamentous fungus has been demonstrated, identification is in fact not necessary for effective treatment. However, as many fungi as possible were identified in the clinical parts of this study to determine if certain fungi consistently showed resistance to the agents being used. In vitro sensitivity testing showed *Aspergillus* spp. tended to be more resistant to 0.2% chlorhexidine, 3 cases in London (appendix-2), and 6 cases in EITC, Chittagong, Bangladesh (appendix-13), altogether 9 cases being resistant. Only one case of *Fusarium* sp. was resistant to 0.2% chlorhexidine (appendix-2). No other fungi were found to be resistant to 0.2% chlorhexidine. Three *Aspergillus* spp. were resistant to 5% natamycin (appendix-3), 7 *Aspergillus* spp., 6 *Fusarium* spp. and 2 *Curvularia* spp. were resistant to 2.5% natamycin (appendix-13). A total of 10 *Aspergillus* spp. were resistant to natamycin. *Fusarium* spp. were more likely to be resistant to 1% econazole, 1 case in London (appendix-6) and 6 cases in the EITC, Chittagong, Bangladesh (appendix-13), altogether 7 cases. One *Cladosporium* sp. (appendix-6) and 1 *Aspergillus* sp. (appendix-13) were resistant to 1% econazole. No other fungi were found to be resistant to 1% econazole. Here resistant means no zone of inhibition.

The in vitro results showed that chlorhexidine has a dose related response. With the increase of concentration the response increases up to 0.2%. In the cases of Brolene and PHMB the number of negative responses were 54 out of 95 and 73 out of 95 respectively which is very high. But with either of them the mean diameter of inhibition in positive cases is greater than chlorhexidine. When they work, they work well. From the clinical point of view Brolene and PHMB were not tested further because of their high number of negative responses. Povidone iodine showed effective

inhibition in all concentrations in vitro but showed disappointing results in vivo. In vitro sensitivity tests are known not always to correlate with in vivo clinical trials. Chlorhexidine sensitivity testing showed reduced mean zone diameters of inhibition in positive cases as compared with all concentrations of povidone iodine alone but was much more effective in the clinical pilot study. The zone diameters of chlorhexidine gluconate and acetate were almost same. In vitro the zone diameters of inhibition produced by 2.5% and 5% natamycin are almost same but in vivo 5% natamycin is more effective than 2.5% natamycin. Econazole proved to be very effective, both in vitro and clinically, but it is not easily available and is expensive.

6.2. Characteristics of patients

As mycotic keratitis is very common in tropical poor developing countries and fungal keratitis affects poor agriculture workers, so the use of a drug such as econazole is not feasible.

Males from 30 to 50 years of age are more affected than any other age groups. Outdoor workers are more affected than extreme age groups who are not working outside and are not prone to develop trauma. More than 50% of patients give a history of trauma, although some patients some times forget about trivial traumas. As people were more aware of eye care, there were fewer patients who used steroids and native medicines prior to corneal scrapings.

6.3. The clinical results

In the pilot study of treatment, it is shown that chlorhexidine 0.2% may be superior to povidone iodine 5% and equal to econazole 1% in producing cure of ulcers. The cured ulcer were 91%, 20% and 87.5% in chlorhexidine 0.2%, povidone iodine 5% and econazole 1% respectively. Povidone iodine was disappointing; out of 5 ulcers only one was cured.

In the randomised trial of different concentrations chlorhexidine and natamycin 5%, it is shown that chlorhexidine may be superior to natamycin 5% and the effect appears to improve with increasing concentration up to 0.2%. It is possible that 0.3% or even higher concentrations would give superior results without toxicity to the cornea, but this has not been tested clinically. The proportions cured at 21 days with chlorhexidine 0.2% and natamycin 5% were 83.3% and 50% respectively. The relative efficacy was 1.67, indicating that in the sample, chlorhexidine 0.2% was 1.67 times more effective

in producing cure within 21 days. The approximate 95% confidence limits for the relative efficacy are 0.88 - 3.14. This suggests that ***at worst*** chlorhexidine should have 88% of natamycin's efficacy.

In the randomised trial of chlorhexidine 0.2% and natamycin 2.5%, it is shown that chlorhexidine 0.2% may be superior to natamycin 2.5% and the proportions cured in non-severe ulcers at 21 days were 66.66% and 38.46% respectively. The efficacy in producing favourable outcome at day 5 were 88.6% and 52.5% in chlorhexidine 0.2% and natamycin 2.5% respectively. The relative efficacy was 1.68. The efficacy in producing favourable outcome at day 5, in ulcer size more than 12 sq. mm. was more convincing. Improvement was seen in 92.9% and 33.3% with chlorhexidine 0.2% and natamycin 2.5% respectively. Although none of them were cured at 21 days, 3 were cured by chlorhexidine 0.2% beyond 21 days and none by natamycin 2.5%. The relative efficacy was 2.79, indicating that in the sample, chlorhexidine 0.2% was 2.79 times more effective in producing a favourable outcome at day 5, in the case of severe ulcers.

The patients' tolerance was good. The drugs were not discontinued in any patient due to allergy or any other problems. No patient complained about burning, itching or tearing due to any one of these medications. In one patient in Bangladesh punctate epitheliopathy of the cornea was seen with chlorhexidine 0.2%. It appeared that the medication had been given more frequently than advised and the staining punctate lesions disappeared when the frequency of administration was reduced to the correct 3 hourly regime. There were no signs of drug intolerance like excoriation and discoloration of skin or contact dermatitis etc. after 3 weeks of application. No symblepharon was noticed, no evidence of follicles or papillary hypertrophy, no other untoward systemic complications were found even after 3 months follow up.

6.4. Properties of chlorhexidine

Chlorhexidine is effective against fungal keratitis, it has already been recommended for the treatment of Acanthamoeba keratitis and in vitro it shows good response against bacteria. It is well known to be effective against a range of Gram positive and Gram negative organisms. It is being used for the treatment of Acanthamoeba keratitis[39]. It is effective against Chlamydia trachomatis[23]. Chlorhexidine digluconate is already used as a preservative in many eye preparations and is therefore approved for use in the human eye. It has been used in several clinical situations for about 40 years. These include sterilisation of the skin, prevention of sepsis in wounds and burns, prevention

of urinary tract infections, especially in catheterised patients, and antisepsis in practical obstetrics, including vaginal washing with 0.2% chlorhexidine solution. Chlorhexidine is regarded as the most effective antimicrobial for oral use. The standard preparation is a 0.2% mouth wash, and it has been available for more than 20 years in some countries. The extensive literature on its antimicrobial properties, applications and safety has been reviewed by Denton[16,79].

When chlorhexidine was first applied to the sterilisation of soft contact lenses, a number of studies of possible toxicity to animal eyes were carried out. For example, Gasset and Ishii found no detectable changes from applications of solutions up to 2% to rabbit eyes twice daily for 7 days[35]. Aqueous chlorhexidine solutions were evaluated for retardation of epithelial regeneration after experimental corneal abrasions[59]. While irrigation with concentrations of 2% or 4% significantly slowed the healing rate, concentrations of 1% or less did not statistically slow healing. It is important to stress that some preparations of chlorhexidine may contain detergents. These should be avoided as they are irritant to the eye.

It is suggested that if fungi are seen on Gram staining or potassium hydroxide mount, chlorhexidine might be a useful first line agent. At the same time cultures could be set up for sensitivity testing. If no improvement is made or sensitivity testing indicates likely resistance, a change could be made to other antifungal agents. It is appreciated that this method is not standardised, but it may be of use in developing countries by the very nature of its simplicity, to give a general indication of the likely susceptibility of fungal isolates from eyes.

In considering chlorhexidine digluconate as a treatment for fungal keratitis in developing countries, where mixed infections may be common and laboratory facilities are not usually available, an advantage will be its wide antimicrobial activity. Chlorhexidine may be recommended as a single antimicrobial agent against bacteria, fungus and Acanthamoeba in situations where specific antibiotics or antifungal agents cannot be obtained.

6.5. Future work

1. A clinical trial of chlorhexidine as a primary treatment against any suppurative keratitis, including bacteria, would be valuable for the situation in developing countries. The second arm of the trial could use a combination of an antibiotic and specific antifungal agent.

2. It would be valuable to assess the use of chlorhexidine by paramedical workers as a prophylaxis in minor injuries or infections, to prevent severe corneal ulcers.

7.0. ACKNOWLEDGEMENTS

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8.0. REFERENCES

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9.0 APPENDICES

Appendix no. 1.

Literature review of micro-organisms.

Sl. no..	Reference	Fungus	Bacteria
1.	Rahman MM., Management of fungal corneal ulcer. Trans. Ophthalmic. Soc. Bangladesh. 1981; 9: 12-19. [58] Islam Eye Hospital, Dhaka, Bangladesh. Period 1979 to 1980. Total 508 cases.	Fungus-22.64%. (115 cases) Aspergillus (all)	
2.	Williams G., Billson F., Hussain R., Howlader SA., Islam N., McClellan K. Microbiological diagnosis of suppurative keratitis in Bangladesh. Br. J. Ophthalmol. 1987;71:315-321[44] Chittagong Eye Infirmary and Training Complex, Bangladesh. August 1983 &84. Total 33 cases, Culture positive 21 cases. Total identified cases-21. .	Fungus-one-third (7 cases.) A. fumigatus-1. A. ochraceus-1. Fusarium solani-1. Unidentified filamentous fungi-4 33.33%.	Bacteria-two-third. (14 cases.) Strept. pneumoniae Ps. aeruginosa 66.67%.

3.	Dunlop AAS; Wright ED; Howlader SA; Islam N.; Hussain R., Suppurative corneal infection in Bangladesh[45] Chittagong Eye Infirmary & Training Complex, Bangladesh Total 142 cases. Culture positive 116 cases. No organisms seen on gram stain & culture was negative in 15 cases (10.7%). 89 cases showed consistent microscopy & culture. 27 cases showed growth on culture but not seen on gram stain. 11 cases organisms seen on gram stain but culture was negative.	Fungus-35.9%, (51 cases) Aspergillus-19,(13%,). A. fumigatus. A. flavus. Fusarium -10,(7%.). F. solani. F. dimerum. Curvularia- 9,(6%). C. fallax. Lasiodiplodia . theobromae-2. Scedosporium-1. Epicoccum-1. Cylindrocarpon vaginae-1. Candida albicans-1. Dichotomophthropsis nymphaerum-1. Fungal hyphae seen-3. Lost-3.	Bacteria-53.5%, (76 cases.) Pseudomonas-24% Strept. pneumoniae-17%
	Total identified cases-127.	40.16%	59.84%.
4.	Williams G., McClellan K., Billson F. Suppurative keratitis in rural Bangladesh: the value of Gram stain in planning management. International Ophthalmol. 1991;15:131-135[46]. Chittagong Eye Infirmary & Training Complex Bangladesh. Total 127 cases. 107 cases was culture positive. 89 cases show both Gram stain & culture positive. In 20 cases (15%) gram stain & culture was negative. 18 cases were gram negative but culture positive. Total identified cases-107	Fungus 40.2%(Isolates) (43 cases.) Aspergillus-21. A. fumigatus. A. flavus. Fusarium-12. Lasidoplodia-2. Acremonium-1. Curvularia-1. Dichromophoropsis-1. Fungi (Unidentified)-5.	Bacteria 59.8%(Isolates) (64 cases.) Strept. pneumoniae Pseudomonas

5.	Liesegang TJ., Forster RK., Spectrum of microbial keratitis in South Florida. Am. J. Ophthalmol.1980; 90: 38-47[47] Between 1 January 1969 to 31 December 1977. .(9 Years) South Florida, USA. Total 663 cases, In 371 cases organisms were isolated, In 292 cases organisms were not identified. In 16 negative cultures definite organisms were seen with Gram stain & Giemsa stain.	Fungus- 35%(Isolates) (133 cases) Fusarium-82. (61%) F. solani-76 (57%). F. episphaeria-3. F. moniliforme-1. F. nivale-1. F. oxysporum-1. Aspergillus-6. A. fumigatus-3. A. flavus-3. Curvularia-8. C. senegalensis C. verruculosa C. pallescens Acremonium (Cephalosporium)-4 Melanconiales (Colletotrichum atramentum)-3. Alternaria-2. Lasiodiplodia theobromae-5. Penicillum-4. Paecilomyces-2. Petriellidum boydii-2 (Allescheriaboydii)- Geotrichum candidum.-1. Myrathecum-1 Volutella-1. Cylindrocarpon-1. Drechslera (Helminthosporium)-1. Cladosporium-1. Candida albicans-10..	Bacteria . 65%(Isolates) (238 cases). Ps. aeruginosa-31% Staph. aureus. Strept. pneumoniae Staph. epidermidis Proteus Corynebacterium.

6.	Robert H. Rosa,Jr., MD. Darlene Miller, MA., Eduardo C. Alfonso, MD. The Changing Spectrum of Fungal Keratitis in South Florida. Ophthalmology, Volume 101, November 6, June 1994[48] Bascom Palmer Eye Institute, Miami,. Florida. Between January 1982 & January 1992. (10 Years). (Four new Fungi not described previously in South Florida e.g. C.parapsilosis, A.terreus, C.tropicalis, and Trichosporon beigellii)	Total 125 Fungal keratitis. Fusarium-79.(62.2%) F. oxysporium-47. (37%). F. solani-30 (23.6%) F. moniliforme-1. (0.8%) Unspecified-1.(0.8%) Candida-16.(12.5%) C. parapsilosis-11. C. albicans-4. C. tropicalis-1. Curvularia-11(8.7%) C. senegalensis C. verruculosa. Aspergillus-5 (4%) A. terreus-2. A. fumigatus-1. A. flavus-1. A. glaucus-1. Paecilomyces-4 (3.2%) Acremonium-3 (2.5%) Cylindrocarpon-2 (1.6%) Lasiodiplodia theobromae-2(1.6%) Petriellidum boydii-1 Melanconiales -1. Drechslera-1 Rhizopus-1. Trichosporn beigellii-1
	One study from South Florida between 1959& 1977, total 19 years.	Fusarium sp. accounted for 76% of fungal keratitis. Fusarium solani-29%.
7.	Thomas PA., Keratomycosis(mycotic keratitis) In: Hay RJ., ed. Bailliere's Clinical Tropical Medicine and Communicable Diseases International Practice and Research. Tropical Fungal Infections. Bailliere Tindall London 1989 Vol. 4. No. 1 pp 269-285.[54] Institute of Ophthalmology Jesoph Eye Hospital, Tiruchirapalli,_India.	Fungus-30% Aspergillus Fusarium Curvularia Acremonium Penicillium

8	McCurrach FE., MBBS, and Taylor HR, MD., FRCAO; Infectious keratitis; Current opinion in Ophthalmology, 1992, 3: 458-465.[14] India.	Fungus-50%
9.	Rosa RH., Jr., MD. Miller D., MA., Alfonso EC., MD, The Changing Spectrum of Fungal Keratitis in South Florida, 0phthalmology Volume 101, November 6, June 1994.[48] India	Aspergillus sp.(27%-64%) Fusarium sp. (6%-32%). Penicillium sp. (2%-29%).

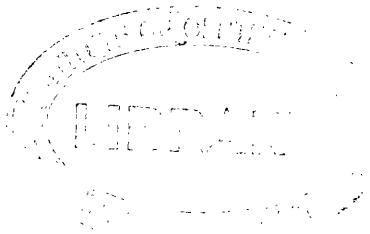
		20.98%	
10.	Upadhyay MP., Karmacharya PCD., Koirala S., Tuladhar NR., Bryan LE., Smolin G., Whitcher JP., Epidemiologic characteristics, predisposing factors and etiologic diagnosis of corneal ulceration in Nepal. Am. J. Ophthalmol. 1991; 111: 92-99[8] Tribhuban University Teaching Hospital, Kathmandu Nepal., Between September 1985 & August 1987. Total cases-405. Culture positive cases-324. Culture negative cases-81. Pure bacterial growth-256. Pure fungal growth-27. Mixed bacterial & fungal growth-41.	Fungus- (68 cases.) Pure fungus-6.7% Aspergillus-31 (47%). A. fumigatus A. flavus A. restricuts A. ustus A. versicolor A. sydowii A. terreus A. oryzae Candida-9 (13.2%). C. krusei C. tropicalis C. pseudotropicalis C. parapsilosis C. guilliermondii Geotrichum candidum. Fusarium-8 (11.7%). F. oxysporum F. solani F. heterospermum F.nivale F.sporotrichoides F.tabacinum Cladosporium-4. C. chlorocephalum C. cladosporoides C. sphaerospermum Mucor sp. -3. M. globosus M. mucedo M. racemosus Lasiodiplodia-3. Curvularia-2, Collectotrichum-1. Nigrospora --1. Phialophora-1. Penicillium-1. Rhizopus-2. Sporotrichum-1.	Bacteria-73.3% (297 cases) Pure bacteria-63.2% Strept. pneumoniae Staph. epidermidis Staph. aureus Corynebacterium xerosis Pseudomonas.
	Total identified cases-324.	21%	79%

11.	Ormerod LD. Causation and management of microbial keratitis in Subtropical Africa. Ophthalmology 1987; 94: 1662-1668[55] St. John's Eye Hospital, Soweto, South Africa. Between 1 March 1981 & 31 January 1982. (11 month). Total 120 patients with 131 episodes, Culture positive-85 cases. Culture negative-28 cases. Culture not available-12 cases. Culture not done-6 cases. Total identified cases-85.	Fungus-3.53% (3 cases) F. solani.-1 Aureobasidium pululans-1 Dichotomophthora portulacae-1.	Bacteria-96.47% (82cases). Staph. aureus Streptococcus Pseudomonas H. influenzae Proteus Klebsiella Enterobacter.
12.	Carmichael TR., Wolpert M., Koornof HJ. Corneal ulceration at an urban Africa hospital. Br. J. Ophthalmol. 1985; 69: 920-926[56] St. John's Eye Hospital, Baragwanath Hospital Soweto, South Africa. Between July 1982 & June 1983. Total 283 Corneal ulcer.(from 274 patients.) Micro-organisms isolated from 87 Eyes. Total identified cases-87	Fungus-7% (6 cases.) Curvularia-2 A. flavus-1 Fusarium-1 Candida-1 Phomaeupyrena-1	Bacteria-93% (81 cases.) Strept. pneumoniae Staph. aureus Ps. aeruginosa Moraxella
13.	Coster DJ., Wilhelmus K., Peacock J., Jones BR., Suppurative keratitis in London. VIth Congress of the European Soc. of Ophthalmol. 1981: pp 395-398.[57] Moorfields Eye Hospital, London. Between April 1978 & November 1979. Total 67 cases. In 41 cases organisms were found. In 26 cases no organisms were found. In 26 Gram negative 3 yield growth on culture. In 41 Gram positive 3 does not yield growth on culture. Total identified cases-41	Fungus-5%. (2 cases.) Candida-1 Cladosporium-1	Bacteria-95% (39 cases.) Staph. aureus. Strept. pneumoniae. Ps. aeruginosa Moraxella

14.	Asbell P., Stenson S. Ulcerative Keratitis; Survey of 30 years Laboratory Experience. Arch. Ophthalmol. 1982; 100: 77-80[12] New York University-Bellevue Medical Center.. Between 1950 to 1979 Total 30 Years. Total 677 cases. Organisms were identified from 547 cases. by Gram stain & culture. Positive cultures were obtained in 494 cases.	Fungus -1.7%. (only 9 cases) Candida-5 Fusarium-2 Nocardia-1 Cryptococcus-1 New York-1% Texas-17%. Florida-35%.	Bacteria -98.3% (538 cases.) Staphylococcus 48% Moraxella 16% Pseudomonas 8% Strept. pneumoniae 8% Proteus 2% and others.
15.	Gugnani HC., Talwar RS, Njoku-obi ANU, and Kodilinye HC; Mycotic Keratitis in Nigeria, A study of 21 cases; Brit. J. of Ophthal. (1976) 60, 607.[40] The study was limited to cases in which the corneal ulcers were strongly suggestive of a fungal infection. Total 26 patients. Fungus cultured in 21 patients.	Fungus (21 cases) Fusarium solani-12 A. fumigatus-4 A. flavus-1 Penicillium citrinum-2 P. expansum-1 Penicillium sp.-1	
16.	Cheung J., MD., Slomovic AR, MD, FRCSC; Microbial aetiology and predisposing factors among patients hospitalised for corneal ulceration, Can J. 0phthalmology-vol. 30, no. 5, 1995, 151-255[6]. The Toronto hospital. Total cases-95 Organisms grown-60 No growth-35	Fungus-0%	Bacteria-100% Coagulase-negative staphyloccus-30% Staph. aureus-23% Strep. pneumoniae-12% Strep. α hemolyticus-3% Gr. A Streptococcus-2% Enterococcus-2% Moraxella-7% Pseudo. aeruginosa-12% Proteus mirabilis-3% Klebsiella-3% Haemoph. influenzae-3% Serratia marcescens-2% Citrobacter diversus-2% E. coli-2% Mycobact. chelonae-2% Propionibacterium acnes-2%

17.	Tsiligianni AK, MD., Alfonso E., MD., and Forster RK, MD; Ulcerative Keratitis Associated With Contact Lens Wear, American Journal of Ophthalmology 108:64-67, July, 1989[51]. Bascom Palmer Institute, Miami, Florida. Total no. of cases-658 Contact lens wearers-196 Non-contact lens wearer-462 Culture positive-349 No cases of fungal keratitis were found in the contact lens wearer group.	Fungus-11.74% Fusarium sp-23 Curvularia sp-5 Others-12 Mixed-1	Bacteria-88.25% P. aeruginosa-132 Pseudomonas sp-5 Serratia marcescens-20 Proteus mirabilis-6 Morganella morganii-4 H. influenzae-5 N. gonorrhoea-3 Staph. aureus-42 Staph. epidermidis-18 Strep. pneumoniae-12 Streptococcus sp-10 Bacillus sp-3 Gram positive rods-12 Gram positive anaerobes-7 Other gram negative-18 Mixed-11
18	Ormerod LD, MD., Hertzmark E, MA., Gomez DS., BS., Stabiner RG., MS, Schanzlin DJ., MD., Smith RE., MD; Epidemiology of Microbial Keratitis in Southern California; Ophthalmology, October 1987, vol. 94 no. 10[52]. Southern California. Total cases-242 Culture-positive-186 Polymicrobial-62 Two organisms-52 Three organisms-10 Four organisms-1 Total no. of bacterial isolates-240 Total no. of fungal isolates-20	Fungus-10% C. albicans-9 Penicillium-3 Helminthosporium-3 Alternaria-2 Scopulariopsis-1 Cladosporium-1 A. flavus-1 Houston-17%. San Francisco-0% Boston-0%	Bacteria-90% Staph. aureus-41 Coagulase-negative staphyloccus-54 Strep. pneumoniae-27 Alpha-streptococci-17 Beta-streptococci-13 Gama-streptococci-4 Microaerophilic streptococci-1 Moraxella-14 Acenetobacter-2 Branhamella catarrhalis-1 B. subtilis Clostridium sp-1 P. aeruginosa-35 Pseudomonas sp-2 Proteus sp-8 E. coli-5 Klebsiella sp-3 Serratia sp-3 Citrobacter sp-2 Enterobacter sp-2 Alcaligenes faecalis-1 Aeromonas hydrophila-1 Eikenella corrodens-1 H. influenzae-1

19.	Hagan M., Wright E., Newman M., Dolin P., and Johnson G.; Causes of Suppurative Keratitis in Ghana; [41]	Fungus alone-49.12% Mixed-56.14%		Bacteria alone-43.85% Mixed-50.87%
	Total no. of patients-199. Nothing cultured-85 cases. Organisms cultured-114 patients.	F. solani-6 F. dimerum-1 Fusarium sp.-27 A. fumigatus-1 A. flavus-5 A. terreus-1 Aspergillus sp. -3 Pseudallescheria boydii-1 Cladosporium sp.-4 L. theobromiae-6 Trichosporon capitatum-1 Nigrospora sp-1 Candida parapsilosis-1 Curvularium fallax-2 Acremonium sp.-1 Phoma sp.-1 Dichotomophthoropsis sp.-1 Unidentified fungi-2	S. pneumoniae-8 Streptococcus sp-3 Enterococcus faecalis-1 Corynebacterium sp-3 S. aureus-4 S. epidermidis-14 Propionibacterium acnes-1 Moraxella sp-4 H. influenzae-1 N. gonorrhoeae-2 Neisseria sp-1 P. aeruginosa-16 Pseudomonas sp-1 Enterobacter cloacae-2 Vibrio metschnikovii-1 Alcaligenes sp-1
	Single organism cultured-103 cases. Gram positive & Gram negative-3 cases. Gram positive & fungus-4 cases. Gram negative & fungus-2 cases. Gram positive, Gram negative & 1 fungus-1 Gram positive, Gram negative & 2 fungus-1			
	Single bacteria-50 Mixed-8 Total-58			
	Single fungus-56 Mixed-8 Total-64.			



Appendix-2

Results of antifungal drug sensitivity tests against fungi from Aravind Eye Hospital, India done in UK.

Sl.no.	Name of Species.	0.02% chlorhexidine (zone diameter in mm)	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine + 0.05% Brolene (zone diameter in mm)	0.2% chlorhexidine + 2.5% povidone-iodine (zone diameter in mm)
695	F. solani	00	01	03	01	03	05
712	F. solani	13	00	02	03	03	05
1372	F. dimerum	05	07	08	08	23	12
393	F. dimerium	07	08	08	07	22	16
396	Fusarium sp.	06	09	07	07	25	08
501	Fusarium sp.	00	00	00	04	04	08
503	Fusarium sp.	02	03	01	02	00	05
505	Fusarium sp.	03	06	04	04	04	07
1120	Fusarium sp	06	08	06	06	23	06
1382	Fusarium sp.	00	05	02	02	02	05
1391	Fusarium.sp.	00	02	02	02	01	05
1397	Fusarium sp.	03	04	02	03	01	05
1401	Fusarium sp.	03	04	00	00	00	05
719	A. fumigatus	00	00	02	02	03	04
735	A. fumigatus	00	00	00	03	03	04
736	A. fumigatus	00	00	00	03	04	05

Appendix-2

Results of antifungal drug sensitivity tests against fungi from Aravind Eye Hospital, India done in UK.

SL. NO.	Name of species.	0.02% chlorhexidine (zone diameter in mm)	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine + 0.05% Brolene (zone diameter in mm)	0.2% chlorhexidine + 2.5% povidone-iodine (zone diameter in mm)
738	A. fumigatus	00	00	00	03	03	02
753	A. fumigatus	00	00	02	03	03	03
758	A. fumigatus	00	00	00	03	03	03
186	A. flavus	N.G	N.G	00	00	00	03
721	A. flavus	00	00	02	01	01	04
737	A. flavus	00	00	00	00	00	02
755	A. flavus	00	01	00	03	00	03
1420	A. flavus	00	00	00	00	00	02
729	A. terreus	00	02	04	04	04	05
219	Coelomycete	04	05	03	06	05	07
387	Coelomycete	00	00	03	03	02	12
416	Coelomycete	06	08	11	16	18	18
492	Coelomycete	01	05	00	05	05	13
507	Coelomycete	03	08	03	06	10	04
518	Coelomycete	00	00	02	03	02	05
733	Coelomycete	00	00	00	04	03	03

Appendix-2

Results of antifungal drug sensitivity tests against fungi from Aravind Eye Hospital, India done in UK.

SL. NO.	Name of species	0.02% chlorhexidine (zone diameter in mm)	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine + 0.05% Brolene (zone diameter in mm)	0.2% chlorhexidine + 2.5% povidone-iodine (zone diameter in mm)
496	Exserohilum sp.	03	05	00	05	05	12
669	Exserohilum sp.	00	03	04	01	01	05
690	Exserohilum sp.	00	00	03	02	02	08
716	Exserohilum sp.	00	01	02	02	02	05
205	Acremonium sp.	00	01	02	04	04	05
322	Acremonium	02	04	00	06	08	12
340	Acremonium sp.	00	03	05	05	05	11
454	L.theobromae	03	03	06	08	07	10
484	L. theobromae	01	04	00	06	06	04
726	L. theobromae	05	06	02	09	09	07
461	Alternaria sp.	00	00	N.G	09	12	N.G
662	Alternaria sp.	00	01	03	03	03	06
190	Curvularia sp.	N.G	N.G	11	11	14	18
574	Curvularia sp.	08	13	13	09	29	04
234	Cylindrocarpon sp.	N.G	N.G	00	02	02	13
445	Exophiata jeanselmei	07	10	13	12	20	18

Appendix-2

Results of antifungal drug sensitivity tests against fungi from Aravind Eye Hospital, India done in UK.

SL. NO	Name of species	0.02% chlorhexidine (zone diameter in mm)	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine + 0.05% Brolene (zone diameter in mm)	0.2% chlorhexidine + 2.5% povidone-iodine (zone diameter in mm)
307	Scedosporium sp.	07	10	11	09	14	23
519	Gliocladium sp.	00	05	08	09	09	N.G
1381	Drechslera sp.	08	11	10	10	23	12
128		00	04	05	07	23	15
129		00	00	03	06	07	08
145		N.G	N.G	04	07	08	12
213	Graphium sp.	02	03	05	06	06	11
303	Acremonium sp.	N.G	N.G	02	06	06	13
499		03	08	00	05	06	05
676		00	01	02	00	01	06
1318		00	00	09	15	09	N.G
1349		00	04	05	04	04	04
723		00	02	03	02	02	05
1081		08	10	09	13	14	16
1210		03	04	05	05	04	07

Appendix-3

Results of further antifungal drug sensitivity tests against fungi from Aravind Eye Hospital, India done in UK.

SL.NO.	Name of Species.	0.1% Brolene (zone diameter in mm)	1% econazole (zone diameter in mm)	0.02% PHMB. (zone diameter in mm)	1% povidone-iodine (zone diameter in mm)	2% povidone iodine (zone diameter in mm)	5% povidone iodine (zone diameter in mm)
695	F. solani	00	14	00	08	11	07
712	F. solani	00	07	00	06	06	05
1372	F. dimerum	23	11	00	13	17	10
393	F. dimerium	22	11	04	08	08	24
396	Fusarium sp.	24	18	00	07	13	11
501	Fusarium sp.	00	18	00	13	18	13
503	Fusarium sp.	00	15	00	08	15	12
505	Fusarium sp.	00	15	00	17	18	10
1120	Fusarium. sp	23	20	00	13	21	07
1382	Fusarium. sp.	00	16	00	14	16	05
1391	Fusarium. sp.	00	08	00	08	10	04
1397	Fusarium sp.	00	09	00	09	13	00
1401	Fusarium sp.?	00	28	00	13	18	10
719	A. fumigatus	00	14	00	05	06	07
735	A. fumigatus	00	11	00	00	00	00
736	A. fumigatus	00	12	00	07	07	05

Appendix-3

Results of further antifungal drug sensitivity tests against fungi from Aravind Eye Hospital, India done in UK.

SL.NO.	Name of Species.	0.1% Brolene (zone diameter in mm)	1% econazole (zone diameter in mm)	0.02% PHMB (zone diameter in mm)	1% povidone-iodine (zone diameter in mm)	2% povidone-iodine (zone diameter in mm)	5% povidone iodine (zone diameter in mm)
738	A. fumigatus	00	13	00	00	04	03
753	A. fumigatus	00	16	00	05	05	00
758	A. fumigatus	00	12	00	00	00	00
186	A.flavus	00	13	00	03	05	09
721	A. flavus	00	12	00	03	04	06
737	A. flavus	00	12	00	00	00	05
755	A. flavus	00	10	00	00	00	06
1420	A. flavus	00	10	00	04	06	05
729	A. terreus	00	14	00	02	04	06
219	Coelomycete	00	13	00	00	00	11
387	Coelomycete	00	14	00	05	08	13
416	Coelomycete	16	38	18	09	13	19
492	Coelomycete	00	15	00	14	18	16
507	Coelomycete	00	17	00	00	04	06
518	Coelomycete	00	13	00	13	15	11
733	Coelomycete	00	14	00	07	07	08

Appendix-3

Results of further antifungal drug sensitivity tests against fungi from Aravind Eye Hospital, India done in UK.

SL .NO.	Name of Species.	0.1% Brolene (zone diameter in mm)	1% econazole (zone diameter in mm)	0.02% PHMB (zone diameter in mm)	1% povidone-iodine (zone diameter in mm)	2% povidone-iodine (zone diameter in mm)	5% povidone -iodine (zone diameter in mm)
496	Exserohilum sp.	00	26	00	11	14	15
669	Exserohilum sp.	00	13	00	13	16	10
690	Exserohilum sp.	00	18	00	15	23	12
716	Exserohilum sp.	00	15	00	13	13	08
205	Acremonium sp.	03	12	00	13	17	14
322	Acremonium	03	27	00	05	20	31
340	Acremonium sp.	00	15	03	11	17	14
454	L. theobromae	01	15	03	00	04	18
484	L. theobromae	05	20	00	00	03	06
726	L. theobromae	00	17	00	00	00	05
461	Alternaria sp.	06	N.G	N.G	10	15	N.G
662	Alternaria sp.	00	17	00	13	18	05
190	Curvularia sp.	12	21	07	08	18	19
574	Curvularia sp.	28	27	05	05	11	12
234	Cylindrocarpon sp.	00	14	00	00	18	31
445	Exophiata jeanselmei	22	18	08	11	13	13

Appendix-3

Results of further antifungal drug sensitivity tests against fungi from Aravind Eye Hospital, India done in UK.

SL. NO.	Name of Species.	0.1% Brolene (zone diameter in mm)	1% econazole (zone diameter in mm)	0.02% PHMB (zone diameter in mm)	1% povidone-iodine (zone diameter in mm)	2% povidone-iodine (zone diameter in mm)	5% povidone-iodine (zone diameter in mm)
307	Scedosporium sp.	07	06	13	13	13	20
519	Gliocladium sp.	09	15	00	08	13	30
1381	Drechslera sp.	23	22	07	07	08	00
128		15	23	08	18	18	22
129		00	15	00	13	21	12
145		00	18	00	05	09	16
213	Graphium sp.	01	20	00	13	16	19
303	Acremonium sp.	00	16	00	15	20	14
499		00	23	08	07	00	08
676		00	13	00	11	11	09
1318		08	N.G	N.G	08	13	N.G
1349		00	00	00	07	09	07
723		00	15	00	08	09	13
1081		08	17	08	08	13	11
1210		11	11	00	05	09	07

Appendix-4

Isolates of fungi from Aravind Eye Hospital, India, in the in vitro sensitivity tests done in UK.

Name of organisms	Total no.	Percentage
Fusarium sp.	13	20.63%
Aspergillus sp.	12	19.04%
Coelomycete sp.	07	11.11%
Exserohilum sp.	04	6.34%
Acremonium sp.	04	6.34%
L. theobrome	03	4.76%
Alternaria sp.	02	3.17%
Curvularia sp.	02	3.17%
Cylindrocarpon sp.	01	1.58%
Exophiata jeanselmei	01	1.58%
Scedosporium	01	1.58%
Gliocladium sp.	01	1.58%
Dreschslera sp.	01	1.58%
Graphium sp.	01	1.58%
Unidentified fungi	10	15.87%

Appendix-5

Results of antifungal drug sensitivity tests against fungi from Ghana done in UK.

SL .NO.	Name of species	0.02% chlorhexidine (zone diameter in mm)	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine + 0.05% Brolene (zone diameter in mm)	0.2% chlorhexidine + 2.5% povidone-iodine (zone diameter in mm)
063	F. solani	00	03	04	06	06	07
156	F. solani	01	01	02	03	03	09
177	F. solani	00	01	02	02	03	05
174	F. solani	00	00	02	03	02	05
133	F. solani	01	03	03	04	03	09
040	Fusarium sp.	00	00	04	05	05	02
052	Fusarium sp.	00	01	04	04	04	07
054	Fusarium sp.	00	00	04	04	05	06
077	Fusarium sp.	00	02	03	03	04	07
078	Fusarium sp.	00	00	02	02	03	09
079	Fusarium sp.	00	00	03	03	03	09
110	Fusarium sp.	01	02	03	04	04	06
111	Fusarium sp.	01	03	07	08	09	05
116	Fusarium sp.	00	00	08	11	10	08
135	Fusarium sp	02	03	03	04	03	10
150	Fusarium sp.	00	02	03	04	04	06
190	Fusarium sp.	00	00	08	13	13	05
205	Fusarium sp.	01	02	03	03	02	07

Appendix-5

Results of antifungal drug sensitivity tests against fungi from Ghana done in UK.

SL. NO.	Name of species	0.02% chlorhexidine (zone diameter in mm)	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine + 0.05% Brolene (zone diameter in mm)	0.2% chlorhexidine + 2.5% povidone-iodine (zone diameter in mm)
144	A. versicolor	00	00	03	03	03	05
146	A. amstelodomi	03	04	03	04	03	09
163	A. niger	00	01	04	03	04	04
172	A. flavus	00	00	02	03	03	07
198	A. terreus	00	00	02	03	23	06
103	Cladosporium sp.	14	16	15	18	25	21
129	Cladosporium sp	00	01	12	13	13	04
115	L. theobrome	05	04	00	06	06	05
168	L. theobrome	04	05	04	06	06	06
130	Nigrospora spp.	00	00	17	18	28	13
166	Curvularia spp.	11	14	15	18	31	16
196	Phoma	03	06	08	08	09	08
037		06	09	09	09	17	09
147		07	08	08	09	15	08

Appendix-6

Results of further antifungal drug sensitivity tests against fungi from Ghana done in UK.

SL. NO.	Name of Species.	0.1% Brolene (zone diameter in mm)	1% econazole (zone diameter in mm)	0.02% PHMB (zone diameter in mm)	1% povidone-iodine (zone diameter in mm)	2% povidone-iodine (zone diameter in mm)	5% povidone-iodine (zone diameter in mm)
063	F. solani	02	18	00	04	08	09
156	F. solani	03	17	00	04	04	10
177	F. solani	00	14	00	07	08	10
174	F. solani	00	08	00	04	04	09
133	F. solani	00	21	00	10	13	15
040	Fusarium sp.	00	00	02	08	11	03
052	Fusarium sp.	00	16	03	05	08	11
054	Fusarium sp.	00	16	00	05	08	12
077	Fusarium sp.	00	17	00	05	10	08
078	Fusarium sp.	02	18	00	00	05	13
079	Fusarium sp.	00	15	00	08	11	13
110	Fusarium sp.	01	16	00	08	13	11
111	Fusarium sp.	04	11	00	05	08	11
116	Fusarium sp.	08	10	00	03	05	05
135	Fusarium sp.	00	17	00	06	13	18
150	Fusarium sp.	01	14	00	08	08	08
190	Fusarium sp.	09	15	00	05	05	08
205	Fusarium sp.	00	13	00	05	04	11

Appendix-6

Results of further antifungal drug sensitivity tests against fungi from Ghana done in UK.

SL NO.	Name Species.	0.1% Brolene (zone diameter in mm)	1% econazole (zone diameter in mm)	0.02% PHMB (zone diameter in mm)	1% povidone-iodine (zone diameter in mm)	2% povidone-iodine (zone diameter in mm)	5% povidone iodine (zone diameter in mm)
144	A. versicolor	01	18	00	04	05	14
146	A.amstelodomi	00	13	00	08	13	23
163	A. niger	02	15	07	00	04	11
172	A. flavus	02	21	00	00	03	16
198	A. terreus	18	18	00	00	04	13
103	Cladosporium spp.	23	31	16	08	13	28
129	Cladosporium spp	05	00	00	08	08	11
115	L. theobrome	00	11	00	04	04	08
168	L. theobrome	05	13	05	00	03	11
130	Nigrospora spp.	28	14	04	00	04	11
166	Curvularia spp.	29	26	13	07	11	14
196	Phoma	05	15	00	11	16	13
037		18	12	00	13	15	18
147		11	09	02	13	21	11

Appendix-7

Isolates of fungi from Ghana in the in vitro sensitivity tests done in UK.

Name of organisms	Total no. of fungi	Percentage
Fusarium sp.	18	56.25%
Aspergillus sp.	05	15.62%
Cladosporium sp.	02	6.25%
L. theobrome	02	6.25%
Nigrospora sp.	01	3.12%
Curvularia sp.	01	3.12%
Phoma sp.	01	3.12%
Unidentified fungi	02	6.25%

Appendix-8

Results of in vitro sensitivity tests of fungi in the randomised trial of different concentrations of chlorhexidine and 5% natamycin done in the Aravind Eye Hospital, Madurai, India.

Study no.	Name of species	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)	5% natamycin (zone diameter in mm)
02	Fusarium sp	00	00	08	18
09	Fusarium sp.	00	03	08	11
54	Fusarium sp.	00	03	05	13
58	Fusarium sp.	08	11	13	18
18	Fusarium sp.	02	05	08	19
19	Fusarium sp.	00	00	03	16
46	Fusarium sp./	13	16	16	18
49	Fusarium sp.	03	06	08	23
22	Fusarium sp.	00	00	05	18
28	Fusarium sp.	00	03	06	13
35	Fusarium sp.	05	06	08	10
24	Fusarium sp.	02	05	10	16
38	Fusarium sp.	00	03	06	08
26	Fusarium sp.	04	05	11	15
56	Fusarium sp.	00	03	03	10
29	Fusarium sp.	00	03	06	10
37	Fusarium sp.	00	03	08	13
42	Fusarium sp.	03	03	06	10
47	Fusarium sp.	05	08	10	13

Appendix-8

Results of in vitro sensitivity tests of fungi in the randomised trial of different concentrations of chlorhexidine and 5% natamycin done in the Aravind Eye Hospital, Madurai, India.

Study no.	Name of species	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)	5% natamycin (zone diameter in mm)
03	Aspergillus flavus	00	00	13	15
14	Aspergillus flavus	00	00	03	05
15	Aspergillus flavus	00	00	05	03
33	Aspergillus flavus	00	00	06	13
34	Aspergillus flavus	00	00	03	00
48	Aspergillus flavus	00	00	02	00
13	Aspergillus flavus	00	00	05	00
36	Aspergillus flavus	00	00	08	20
39	Aspergillus fumigatus	00	00	03	18
45	Curvularia sp.	10	13	16	18
25	Curvularia sp.	06	08	11	15
27	Curvularia sp.	08	13	18	23
53	Exserophillum sp.	09	13	16	18
60	Monosporium sp.	08	10	13	18
50	Rhizopus sp.	04	06	08	09
44	Botryodiplodia sp.	08	10	13	23
16	Unidentified	00	05	13	00
17	Unidentified	18	20	28	18
59	Unidentified	08	10	13	05
51	Unidentified	06	11	13	00
12	Unidentified	01	02	07	03

Appendix-9

Results of antibacterial sensitivity tests against most common bacteria done in the Aravind Eye Hospital, Madurai, India

Name of antibiotics	Pseudomonas (zone diameter in mm)	Pneumococcus (zone diameter in mm)	Staphylococcus epidermidis (zone diameter in mm)	Staphylococcus aureus (zone diameter in mm)
0.05% chlohexidine gluconate	08	08	13	09
0.1% chlorhexidine gluconate	10	08	13	13
0.2% chlorhexidine gluconate	15	12	17	14
0.05% chlorhexidine acetate	08	03	16	13
0.1% chlorhexidine acetate	11	04	18	16
0.2% chlorhexidine acetate	13	08	21	18
Gentamycin (well diffusion method)	33	30	18	23
Gentamycin. (disc diffusion method)	30	28	18	20
Chloramphenicol. (well diffusion method)	13	20	31	23
Chloramphenicol. (disc diffusion method)	15	18	30	25
Ciprofloxacin. (well diffusion method)	33	23	28	23
Ciprofloxacin. (disc diffusion method)	35	21	30	23

Appendix-10

Results of antibacterial sensitivity tests against different concentrations of chlorhexidine gluconate done in the Aravind Eye Hospital, Madurai, India.

Name of micro-organisms	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)
Pseudomonas	08	10	15
Klebsiella	08	10	13
Citrobacter freundi	09	10	13
Salmonella typhi	08	09	11
Proteus	11	11	13
Acinetobacter	11	16	18
E. coli	09	10	13
Pneumococcus	08	08	12
Staphylococcus aureus	09	13	14
Staphylococcus epidermidis	13	13	17
Streptococcus α haemolyticus	11	11	15
Streptococcus pyogens	06	08	11
Streptococcus agalactiae	13	13	14

Appendix-11

Results of antibacterial sensitivity tests against different concentrations of chlorhexidine acetate done in the Aravind Eye Hospital, Madurai, India.

Name of micro-organisms	0.05% chlorhexidine (zone diameter in mm)	0.1% chlorhexidine (zone diameter in mm)	0.2% chlorhexidine (zone diameter in mm)
Pseudomonas	08	11	13
Klebsiella	08	10	13
Citrobacter freundi	11	12	16
Salmonella typhi	10	13	15
Proteus	08	11	13
Acinetobacter	16	16	18
E. coli	11	13	13
Pneumococcus	03	04	08
Staphylococcus aureus	13	16	18
Staphylococcus epidermidis	16	18	21
Streptococcus alpha haemolyticus	03	06	10
Streptocuccus pyogens	06	08	10
Streptococcus agalactiae	05	08	11

Appendix-12

Results of antibacterial sensitivity tests against ciprofloxacin, chloramphenicol & gentamycin done in the Aravind Eye Hospital, Madurai, India.

Name of micro-organisms	ciprofloxacin well diffusion method (zone diameter in mm)	ciprofloxacin disc diffusion method (zone diameter in mm)	chloramphenicol well diffusion method (zone diameter in mm)	chloramphenicol disc diffusion method (zone diameter in mm)	gentamycin well diffusion method (zone diameter in mm)	gentamycin disc diffusion method (zone diameter in mm)
Pseudomonas	33	35	13	15	33	30
Klebsiella	28	30	23	23	19	20
Citrobacter freundi	38	33	23	25	23	25
Salmonella typhi	28	30	23	25	19	20
Proteus	33	35	25	25	21	20
Acinetobacter	28	30	28	30	23	23
E. coli	33	35	28	30	23	20
Pneumococcus	23	21	20	18	30	28
Staphylococcus aureus	23	23	23	25	23	20
Staphylococcus epidermidis	28	30	31	30	18	18
Streptococcus alpha haemolyticus	23	25	25	23	23	20
Streptococcus pyogens	23	21	11	08	33	30
Streptococcus agalactiae	33	24	41	40	28	25

Appendix-13

Results of antifungal sensitivity tests in the randomised trial chlorhexidine 0.2% and natamycin 5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Study no.	Name of species	0.2% chlorhexidine (zone diameter in mm)	1% econazole (zone diameter in mm)	2.5% natamycin (zone diameter in mm)
01	Aspergillus sp.	00	15	00
06	Aspergillus sp.	03	13	15
09	Aspergillus sp.	00	11	00
11	Aspergillus fumigatus.		Lost	
12	Aspergillus sp.	00	10	00
14	Aspergillus fumigatus	08	23	18
15	Aspergillus fumigatus	04	23	18
16	Aspergillus fumigatus	00	14	09
17	Aspergillus sp.	09	12	10
20	Aspergillus sp.	03	15	21
21	Aspergillus fumigatus	00	11	00
26	Aspergillus sp.	04	18	13
27	Aspergillus sp.	00	10	00
35	Aspergillus sp.	03	23	18
47	Aspergillus sp.	05	28	18
61	Aspergillus sp.	08	23	14
62	Aspergillus sp.	02	12	00
65	Aspergillus sp.	05	15	13

Appendix-13

Results of antifungal sensitivity tests in the randomised trial chlorhexidine 0.2% and natamycin 5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Study no.	Name of species	0.2% chlorhexidine (zone diameter in mm)	1% econazole (zone diameter in mm)	2.5% natamycin (zone diameter in mm)
67	Aspergillus fumigatus	18	13	13
69	Aspergillus sp.	11	18	13
70	Aspergillus fumigatus	04	00	00
71	Aspergillus fumigatus	13	11	13
04	Fusarium sp.	22	00	00
05	Fusarium sp.	08	25	23
07	Fusarium sp.	14	00	00
19	Fusarium sp.	15	00	08
22	Fusarium sp.	13	10	18
23	Fusarium sp.		No growth on sub-culture	
25	Fusarium sp.	08	20	14
28	Fusarium sp.	10	14	14
29	Fusarium sp.	08	23	18
31	Fusarium sp.	15	18	18
32	Fusarium sp.	11	18	16
34	Fusarium sp.	06	00	00
37	Fusarium sp.	14	28	19

Appendix-13

Results of antifungal sensitivity tests in the randomised trial chlorhexidine 0.2% and natamycin 5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Study no.	Name of species	0.2% chlorhexidine (zone diameter in mm)	1% econazole (zone diameter in mm)	2.5% natamycin (zone diameter in mm)
41	Fusarium sp.	05	16	06
42	Fusarium sp.	13	13	15
43	Fusarium sp.	13	23	18
46	Fusarium sp.	07	15	11
52	Fusarium sp.	23	11	16
54	Fusarium solani	19	14	00
59	Fusarium sp.	03	11	00
60	Fusarium sp.	03	00	00
68	Fusarium sp.	11	00	13
08	Curvularia sp.	18	28	18
24	Curvularia sp.	13	13	15
33	Curvularia	08	06	00
50	Curvularia sp.	15	08	00
53	Curvularia sp.	08	04	00
02	Cylindrocarpon sp.	15	08	10
03	Cylindrocarpon sp.	08	33	25
36	Cylindrocarpon sp.	10	08	06

Appendix-13

Results of antifungal sensitivity tests in the randomised trial chlorhexidine 0.2% and natamycin 5% done in the Eye Infirmary and Training Complex, Chittagong, Bangladesh.

Study no.	Name of species	0.2% chlorhexidine (zone diameter in mm)	1% econazole (zone diameter in mm)	2.5% natamycin (zone diameter in mm)
30	Lasiodiplodia theobromae	05	11	06
44	Lasiodiplodia theobromae	06	11	08
48	Lasiodiplodia theobromae	09	03	03
56	Lasiodiplodia theobromae	13	06	12
45	Coelomycete sp.	11	20	18
51	Drechslera hawaiensis.	08	16	14
57	Colletotrichum dematium	12	04	04
38	Unidentified	05	08	00
40	Unidentified	23	28	21
58	Unidentified	00	21	18
63	Unidentified	15	17	18