**Journal of Basic & Applied Mycology (Egypt) 4 (2013): 13-19**
 

# In-*vitro* response of microbiota isolated from human eye infections to some antibiotics

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Received 13/4/2013, Accepted 3/6/2013

**Abstract:** Five antifungal drugs commonly used in the treatment of fungal eye diseases were tested against the common fungi associated with eye infections using standard agar disk diffusion method. All drugs examined except Floconazole (2%) showed high to moderate antifungal activity. All the 74 bacterial isolates tested against ten antibacterial drugs were affected by Cephotaxime, Ceftriaxone, Ciprofloxacin, Norfloxacin and Tetracycline. Most of drugs tested showed high to moderate effect on Gram-positive bacteria, and weak effect on Gram-negative bacteria, except Ciprofloxacin and Norfloxacin showed high or moderate effect against *Pseudomonas aeruginosa*. Treatment with proper antifungal and/or antibacterial agents will be of great advantage and ophthalmologists should be aware of the drug of choice to be used.

**Key words:** Eye infection, drugs, Antibacterial, antifungal, fungi, bacteria.

## Introduction

The concentration of the drug applied to the eye may be increased by the preparation of fortified eye drops. Frequent topical application of drops is a useful means of achieving therapeutic levels in the eye. Ointments and subconjunctival injections may prolong the contact time between the antifungal and the corneal and conjunctival tissue (Aminov 2009, Dash *et al.* 2011 and Kavitha *et al*. 2012). Only Amphotericin B and Miconazole are available as ophthalmic ointments (Friedberg *et al*. 1991 and Mauger and Craig 1994). Miconazole was reported to be useful for the therapy of mycotic keratitis (Foster 1981 and Fitzsimons and Peters 1986). Intravenous and topical Miconazole and Ketoconazole administrations have also been reported to be useful in therapy of keratitis due to *Scedosporium apiospermum* (Nunery *et al*. 1985 and Ruben 1991).

Oral Fluconazole therapy has been used with success in some cases, treating mycotic keratitis (Thakar 1994), multifocal choroiditis due to coccidioidomycosis (Cunningham *et al*. 1998), chorioretinitis and iridocyclitis complicating disseminated coccidioidomycosis and endogenous *Candida* endophthalmitis (Luttrull *et al*. 1995). Ketoconazole is currently available as an oral preparation worldwide. Ishibashi (1983) reported that oral ketoconazole therapy was effective with mycotic keratitis. Corneal ulcer due to *Fusarium solani* was treated with Ketoconazole and Miconazole and responded well (Mirza 2005).

Topical antibiotics are used to treat bacterial blepharitis, conjunctivitis, keratitis and external hordeola. The most likely pathogens are *Staphylococcus aureus*, coagulase negative staphylococci, streptococci, *Haemophilus influenza*, coliforms and *P. aeruginosa* (Seal *et al.* 1982). Chlortetracycline is a broad spectrum bacteriostatic antibiotic. Tetracyclines are actively concentrated within phagocytes so that it is useful against intracellular pathogens such as *Chlamydia* (Neu 1978). Chloramphenicol's spectrum of activity covers the majority of ocular pathogens (Seal *et al*. 1982).

Aminoglycosides such as Gentamycin has the broadest antibacterial spectrum, with activity against *P. aeruginosa* (Gwon 1992, Chattopadhyay and Grossart 2010 and Palaniappan and Holley 2010). Grag *et al*. (1999) carried out a survey of 37 ophthalmic centers in the United Kingdom and showed that the majority of used antibiotics are not commercially available. Fortified Gentamycin is frequently combined with Cefuroxime in the treatment of bacterial keratitis (Timewell *et al*. 1983). Vancomycin and Teicoplanin are also useful agents against Gram-positive bacteria while Ceftazidime is used mainly as an anti-pseudomonad. Erythromycin, used in the form of an eye ointment, is an alternative to Tetracycline in the treatment of infections caused by Gram-positive organisms. Also it is used in combination with systemic Tetracycline therapy for *Chlamydia*. Amikacin has been shown to be a useful antibiotic against *Pseudomonas* with multiple drug resistance (Shanmuganathan *et al*. 2005).

The aim of the work is to study the sensitivity of microorganisms, isolated from eye infections in Taiz City, Yemen, towards some pharmaceutical drugs.

## Materials and Methods

### Organisms and culture maintenance

Eighty-five isolates (11 of fungi and 74 of bacteria) recovered from the anterior lid margins and

ulcerated area (blepharitis), conjunctival sac (dacryocystitis) and cornea (keratitis) in Taiz City, Yemen (refer to Abdel-Sater *et al.* 2012), were used in the present study. Fungal isolates were routinely maintained on Sabouraud's dextrose agar and incubated at 28ºC, whereas bacterial isolates were maintained on nutrient agar and incubated at 37ºC and *Streptococcus* spp. were maintained on chocolate agar medium at 37ºC.

**Pharmaceutical agents**

Five types of antifungal drugs, namely Ketoconazole (1%), Clotrimazole (1%), Floconazole (2%), Nystatin (1%) and Miconazole (1%), obtained from the Yemen Commercial Center, were used in this study. Also, ten antibacterial drugs [Cephotaxime (30 µg), Chloramphenicol (30 µg), Gentamycin (10 µg), Amoxicillin (10 µg), Ciprofloxacin (5 µg), Ceftraxone (30 µg), Tetracycline (30 µg), Erythromycin (15 µg), Clindamycin (2 µg), and Norofloxacin (10 µg)] were used.

**Determination of antimicrobial activity**

The standard agar disk diffusion method (Cheesbrough 1992) was adopted and performed as follows: sterile 6 mm filter paper disks (Whatman No.1) were impregnated in newly synthesized pharmaceutical drugs. The disks were then air dried for 1 hour. By using a sterile loop, the fungal or bacterial suspensions were spreaded on the surface of Sabouraud's dextrose agar or nutrient agar and blood agar plates, respectively. The discs saturated with the tested compounds were then placed on the agar surface (5 disks for each plate). Each plate had one control disk impregnated with the solvent. The plates were incubated at 28ºC for one week in case of fungi and at 37ºC for 24-48 hours in case of bacteria. The inhibition zones (in mm) around disks were then measured (Cheesbrough 1992).

## Results and Discussion

Five antifungal drugs commonly used in the treatment fungal eye infection were tested against 11 isolates of the common fungi associated with eye infections (Table 1). All isolates were sensitive to Miconazole (1 %), Clotrimazole (1%), Ketoconazole (1%) and Nystatin (1%), while all isolates tested were resistant to Floconazole (2%). Miconazole (1%) exhibited high activity against all fungal isolates tested. Clotrimazole (1%) had high or moderate effect against isolates of *Aspergillus flavus*, *A. oryzae* and *Candida albicans*, whereas it showed moderate activity towards *A. awamori*, *A. niger* and *Penicillium griseofulvum*. Ketoconazole (1 %) showed moderate activity against most fungal isolates (9 out of 11) whereas it showed high activity against *A. oryzae* isolates and weak activity against *A. niger*. Nystatin (1%) was less effective against the tested isolates, showing moderate effect towards *A. flavus* (2 out of 4) and isolates of *A. niger, C. albicans* and *P. griseofulvum* (one isolate each) and weak effect towards isolates of *A. awamori* and *A. oryzae* (Table 1).

There are relatively few therapeutically useful antifungal agents compared to the large number of antibacterial agents that are available. This is due mainly to the fact that fungi and man are both eukaryotes and most substances that kill or inhibit fungal pathogens are also toxic to the host. Most antifungal agents exploit differences in the sterol composition of the fungal cell membrane. Antifungal agents vary considerably in their spectrum of activity (Kwon-Chung and Bennett 1992 and Midgley and Moore 1998).

In the 1980s, Miconazole was reported to be useful for therapy of mycotic keratitis in two series of patients. In the first series (Foster 1981), Miconazole resulted in resolution of all lesions in seven patients with mycotic keratitis (four cases due to *C. albicans*, two due to *A. fumigatus*, and one due to *A. flavus*). In the other series (Fitzsimons and Peters 1986), Miconazole was used in combination with Ketoconazole to treat 17 patients with mycotic keratitis (eight cases due to *Fusarium* sp., and 4 to *Aspergillus* sp., 3 to *Drechslera* sp. and one of each of *Curvularia* sp. and *Candida* sp.). Topical Miconazole administration has been reported to be useful in therapy of superficial keratitis due to *Scedosporium apiospermum* (*Pseudallescheria boydii*) (Ruben 1991) or as primary therapy (Panda *et al*. 1996). Intravenous administration of Miconazole was reported to result in successful outcomes in patients with *S. apiospermum* orbital infection (Nunery *et al*. 1985).

Ishibashi (1983) reported that oral Ketoconazole therapy was effective in two patients with mycotic keratitis, one case due to *F. solani*, and the other due to an unidentified fungus. Keratitis due to dematiaceous fungi other than *Curvularia* spp. appears to respond to primary therapy with oral and/or topical Ketoconazole and oral Ketoconazole with topical Miconazole (Rosa *et al*. 1994 and Garg *et al*. 2000).

Two doses of Ketoconazole (100 mg per day) with topical Miconazole ointment for 6 weeks have been recommended for treatment of mycotic blepharitis due to *Candida* spp. (Huber-Spitzy *et al*. 1991).

Dacryocystitis due to *C. albicans* resolved with surgery alone in one patient and with surgery and topical Miconazole therapy in another (Purgason *et al*. 1992). Miconazole was totally ineffective in patients with *Fusarium* keratitis (Thomas 2003).

A steady accumulation in both normal corneas and those infected with *C. albicans* was noted when Fluconazole was given in a twice-daily divided dose. The presence of inflammation induced by fungal infection did not influence corneal uptake (O'Day *et al*. 1990). Cryptococcal chorioretinitis can be treated with oral Fluconazole (Agarwal *et al*. 1991).

In the study of Algalibi (2000), Clotrimazole (1%) and Ketoconazole (2%) were effective *in vitro* against all tested fungi but with variable capabilities. Miconazole nitrate (2%), Isoconazole nitrate (1%) and Tioconazole (1%) were also effective against all tested fungi except *Rhizopus stolonifer*. Also, Tioconazole (1%) and Miconazole (2%) exhibited moderate to low activities against *A. niger, A. ochraceus, A. terreus, A.versicolor* and *Trichothecium roseum* as compared with Clotrimazole (1%), Isoconazole nitrate (1%) and Ketoconazole (2%) which showed high activities against tested *Aspergillus* species and *Trichothecium roseum.*

In another *in vitro* study of Abdul-Rahman (2004), Miconazole nitrate (1%) and Ketoconazole (2%) were the most effective antifungal drugs in vitro against all tested fungi but with variable capabilities. Of the tested species, *Penicillium funiculosum*, *A. flavus*, *A. ochraceus*, *P. chrysogenum* and *P. citrinum* were the most resistant fungal species. They showed no inhibition with Griseofulvin and Nystatin, while *F. solani* and *A. niger* showed the most sensitive fungal isolates against tested antifungal drugs.

Also, the different antifungal therapeutic agents containing Terbinafine HCL, Itraconazole, Sertaconazole, Tioconazole, and Clotrimazole were highly effective *in vitro* on *A. flavus*, *A. fumigatus*, *C. albicans*, *Chrysosporium tropicum* and *Scopulariopsis brevicaulis* and less effective on *Geotrichum candidum*, *Trichophyton interdigitale*, *T. mentagrophytes* and *T. rubrum* (Sallam 2006).

Table (1): Effect of some antifungal drugs on fungal isolates commonly recovered from human eye infections (measured as diameter of inhibition zone in mm).

| Species | NIT | Clotrimazole (1%) | | | | Ketoconazole (1%) | | | | Miconazole (1%) | | | | Nystatin (1%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NIP | H | M | W | NIP | H | M | W | NIP | H | M | W | NIP | H | M | W |
| *Aspergillus awamori* | 1 | 1 | - | 1 | - | 1 | - | 1 | - | 1 | 1 | - | - | 1 | - | - | 1 |
| *A. flavus* | 4 | 4 | 2 | 2 | - | 4 | - | 4 | - | 4 | 4 | - | - | 4 | 2 | 2 | - |
| *A. niger* | 1 | 1 | - | 1 | - | 1 | - | - | 1 | 1 | 1 | - | - | 1 | - | 1 | - |
| *A. oryzae* | 3 | 3 | 1 | 2 | - | 3 | 1 | 2 | - | 3 | 3 | - | - | 3 | - | - | 3 |
| *Candida albicans* | 1 | 1 | 1 | - | - | 1 | - | 1 | - | 1 | 1 | - | - | 1 | - | 1 | - |
| *Penicillium griseofulvum* | 1 | 1 | - | 1 | - | 1 | - | 1 | - | 1 | 1 | - | - | 1 | - | 1 | - |
| Total isolates | 11 | 11 | 4 | 7 | 0 | 11 | 1 | 9 | 1 | 11 | 11 | 0 | 0 | 11 | 2 | 5 | 4 |
| % of positive isolates | | 100 | | | | 100 | | | | 100 | | | | 100 | | | |

NIT= number of isolates tested; NIP= number of positive isolates.
H= high effect (more than 6.0 mm); M= moderate effect (3.0-6.0 mm); W= weak effect (less than 3.0 mm).
*Floconazole at 2 % concentration showed no effect against all fungi tested.

The antibacterial effect of the 10 drugs commonly used in treatment of eye diseases against 74 bacterial isolates were tested (Table 2). Most drugs tested had antibacterial effect against numerous bacterial isolates. All bacterial isolates tested were affected by Cephotaxime, Ceftriaxone, Ciprofloxacin, Norfloxacin and Tetracycline, while 98.6% of the isolates were inhibited by Gentamycin, 90.5% by Chloramphenicol and 89.2% by Clindamycin and Erythromycin. On the other side, only 44 isolates (59.5% of total isolates) were inhibited by Amoxycillin. On the other hand, Amoxycillin have no effect on isolates of *Staphylococcus epidermidis, E. coli, Pseudomonas aeruginosa* and *Alcaligenes* sp. Similarly, Chloramphenicol showed no effect on *S. pyogenes* and *P. aeruginosa*, Clindamycin and Erythromycin on *E. coli* and *P. aeruginosa* and Gentamycin on *Streptococcus pyogenes*.

Also, the current data presented in table (2) showed that most of drugs tested had high or moderate effect against Gram-positive bacteria and weak effect against Gram-negative bacteria, except Ciprofloxacin and Norfloxacin which showed high or moderate effect against *P. aeruginosa*. Among the Gram-positive bacteria, it was noticed that *B. cereus* and *S. aureus* were the most sensitive towards most drugs while *S.*

*epidermidis* and *S. pyogenes* were less sensitive. Among the Gram-negative bacteria tested, *Alcaligenes* sp. was the most resistant towards the examined drugs, followed by *E. coli* and *P. aeruginosa*.

McClellan *et al*. (1989) found 95% of culture-proven cases were caused by bacteria and most bacterial isolates were sensitive to Gentamicin.

Insler *et al*. (1991) recommended the use of topical Ciprofloxacin for methicillin-resistant *S. aureus* keratitis. Also, Ciprofloxacin ophthalmic ointment was effective and safe topical antimicrobial agent for the treatment of bacterial keratitis caused by susceptible microorganisms (Wilhelmus *et al*. 1993).

Of 1558 corneal isolates, 478 (30.7%) were not sensitive to Ciprofloxacin. Among the isolates, 355 (32.5%) of the 1091 Gram-positive cocci were not sensitive to Ciprofloxacin, and 2 of the 20 Gram-positive bacilli, 22 of the 165 Gram-negative organisms, and 99 of the 282 actinomycetes and related organisms were not sensitive to Ciprofloxacin (Kunimoto *et al*. 1999).

In the study of Algalibi (2000) most of the tested antibiotics were active *in vitro* against Gram-positive bacteria. Only four drugs showed high inhibitory action towards all tested bacteria (percentage of inhibition between 75%-100% for all bacteria). These antibiotics were Ofloxacin, Ciprofloxacin, Gentamicin and Cephradine. Seven antibiotics exhibited low or no activity against Gram-negative bacteria. These include; Clindamycin, Erythromycin, Neomycin, Penicillin G, Tobramycin, Tetracyclin and Chloramphenicol. *P. aeruginosa* followed by *Serratia marcescens* were the most resistant bacteria against most of the tested antibiotics. On the other hand, *Bacillus cereus* was the most susceptible organism to antibiotics. Also, Abdul-Rahman (2004) found that Ciprofloxacin, Chloramphenicol, Gentamycin and Tetracyclin were the most active drugs *in vitro* against Gram-negative bacteria, while Penicillin, Colistin sulphate and Tobramycin were the lowest active drugs against bacteria. *E. coli* and *Neisseria gonorrhoeae* showed high resistance to all drugs, followed by *Proteus mirabilis* and *P. aeruginosa*, which showed resistance to 12 types of drugs (Abdul-Rahman 2004). Bacterial isolates that were resistant to Ciprofloxacin and Ofloxacin were also resistant to Levofloxacin (Kowalski *et al*. 2001).

Chloramphenicol eye drops were useful for the treatment of Methicillin-resistant *S. aureus* (MRSA) ocular surface infections (Fukuda *et al*. 2002). Lomholt and Kilian (2003) found that the vast majority of eye isolates of *P. aeruginosa* from European countries are fully susceptible to Ciprofloxacin.

Sharma *et al*. (2004) showed that, a significant resistance of *S. aureus* to many antibiotics including Ciprofloxacin and referred to the need for an alternative to Ciprofloxacin monotherapy for the treatment of staphylococcal keratitis.

All *Pseudomonas* isolates (100%) while only 86 % of Gram-negative isolates were sensitive to Ciprofloxacin. *Alcaligenes* sp. was resistant to all antibiotics tested with the exception of Ceftazidime (Briscoe *et al*. 2005). However, no Ciprofloxacin resistance was identified in all *Pseudomonas* isolates tested by Leibovitch *et al*. (2005).

The efficacy of topical Moxifloxacin (0.5%) in the treatment of *P. aeruginosa* keratitis in rabbits suggests a potential value for topical Moxifloxacin as a broad-spectrum agent in the treatment of bacterial keratitis (Aliprandis *et al*. 2005).

Most microorganisms isolated from patients with bacterial keratitis (Coagulase-negative staphylococci, *P. aeruginosa*, *Corynebacterium* spp., *S. aureus* and *Streptococcus* spp.) showed susceptibility to Ciprofloxacin and aminoglycosides (Ly *et al*. 2006).

**Conclusion:** Treatment with a proper antibiotic based on culturing and sensitivity testing will be of great advantage. Also, ophthalmologists should be aware of the drug of choice to be used for eye infection.

Table (2): Effect of some antibacterial drugs on bacterial isolates commonly recovered isolated from human eye infections (measured as diameter of inhibition zone in mm).

| Species | NIT | Amoxycillin (10 µg) | | | | Cephotaxime (30 µg) | | | | Ceftriaxone (30 µg) | | | | Chloramphenicol (30 µg) | | | | Ciprofloxacin (5 µg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NIP | H | M | W | NIP | H | M | W | NIP | H | M | W | NIP | H | M | W | NIP | H | M | W |
| *Staphylococcus aureus* | 40 | 40 | 15 | 25 | - | 40 | - | 40 | - | 40 | 29 | 11 | - | 40 | 30 | 10 | - | 40 | 35 | 5 | - |
| *S. epidermidis* | 20 | - | - | - | - | 20 | 16 | 4 | - | 20 | 13 | 5 | 2 | 20 | - | 17 | 3 | 20 | - | 20 | - |
| *Streptococcus pyogenes* | 1 | 1 | - | 1 | - | 1 | - | 1 | - | 1 | - | 1 | - | - | - | - | - | 1 | 1 | - | - |
| *Bacillus cereus* | 3 | 3 | - | - | 3 | 3 | 3 | - | - | 3 | 3 | - | - | 3 | 2 | 1 | - | 3 | 3 | - | - |
| *Escherichia coli* | 2 | - | - | - | - | 2 | - | - | 2 | 2 | - | - | 2 | 2 | - | - | 2 | 2 | - | 1 | 1 |
| *Pseudomonas aeruginosa* | 6 | - | - | - | - | 6 | - | - | 6 | 6 | - | - | 6 | - | - | - | - | 6 | 4 | 2 | - |
| *Alcaligenes* sp. | 2 | - | - | - | - | 2 | - | - | 2 | 2 | - | - | 2 | 2 | - | - | 2 | 2 | - | 1 | 1 |
| Total isolates | 74 | 44 | 15 | 26 | 3 | 74 | 19 | 45 | 10 | 74 | 45 | 17 | 12 | 67 | 3 | 60 | 4 | 74 | 43 | 29 | 2 |
| % of positive isolates | | 59.5 | | | | 100 | | | | 100 | | | | 90.5 | | | | 100 | | | |

Table (2): Cont.

| Species | NIT | Clindamycin (2 µg) | | | | Erythromycin (15 µg) | | | | Gentamycin (10 µg) | | | | Norfloxacin (10 µg) | | | | Tetracycline (30 µg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NIP | H | M | W | NIP | H | M | W | NIP | H | M | W | NIP | H | M | W | NIP | H | M | W |
| *Staphylococcus aureus* | 40 | 40 | - | 37 | 3 | 40 | - | 40 | - | 40 | - | 34 | 6 | 40 | - | 10 | 30 | 40 | 32 | 8 | - |
| *S. epidermidis* | 20 | 20 | - | 17 | 3 | 20 | - | - | 20 | 20 | - | 16 | 4 | 20 | - | 13 | 7 | 20 | 5 | 15 | - |
| *Streptococcus pyogenes* | 1 | 1 | - | - | 1 | 1 | - | 1 | - | - | - | - | - | 1 | - | - | 1 | 1 | - | 1 | - |
| *Bacillus cereus* | 3 | 3 | 1 | 2 | - | 3 | - | - | 3 | 3 | 2 | 1 | - | 3 | - | 2 | 1 | 3 | 3 | - | - |
| *Escherichia coli* | 2 | - | - | - | - | - | - | - | - | 2 | - | - | 2 | 2 | - | 1 | 1 | 2 | - | - | 2 |
| *Pseudomonas aeruginosa* | 6 | - | - | - | - | - | - | - | - | 6 | - | 6 | - | 6 | 5 | 1 | - | 6 | - | - | 6 |
| *Alcaligenes* sp. | 2 | 2 | - | - | 2 | 2 | - | - | 2 | 2 | - | - | 2 | 2 | - | - | 2 | 2 | - | - | 2 |
| Total isolates | 74 | 66 | 1 | 56 | 9 | 66 | - | 41 | 25 | 73 | 3 | 60 | 10 | 74 | 6 | 23 | 45 | 74 | 43 | 21 | 10 |
| % of positive isolates | | 89.2 | | | | 89.2 | | | | 98.6 | | | | 100 | | | | 100 | | | |

NIT= number of isolates tested; NIP= number of positive isolates.
H= high effect (22.0-27.5 mm); M= moderate effect (16.0-21.0 mm); W= weak effect (8.0-15.0mm).

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