Iranian Journal of Pharmaceutical Research (2008), 7 (2): 131-134
Received: June 2007
Accepted: January 2008

Original Article

Sensitivity Pattern of Bacteria Isolated from Contact Lens Wearers in the Faculty of Pharmacy, Karachi University Student Population

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Abstract

Contact lens wearers are at great risk of developing microbial keratitis because of incorrect usages and unhygienic maintenance of contact lenses. Therefore, the present study was planned to provide data that will be helpful in selecting the anti-microbial to cure microbial keratitis. One hundred bacterial isolates from conjunctiva of contact lenses wearer were isolated, identified and subjected to in vitro antibiotic sensitivity. In vitro sensitivity testing was done by Kirby-Bauer disc diffusion method. Out of forty-one isolates of *Staphylococcus epidermidis* isolated in this study, 82.9% and 75.6% isolates were sensitive to amoxycillin and cephradine respectively, where as isolates sensitive to neomycin, ciprofloxacin and chloramphenicol were found to be 95%, 92.9% and 87.8%, respectively. Thirty-nine isolates of *Pseudomonas aeruginosa* showed sensitivity to amoxicillin (2.6%), cephradine (7.7%), neomycin (69.2%), ciprofloxacin (82%), imipenem (84.4%) and chloramphenicol (28.3%), respectively. Multi-resistant strains of pathogenic, as well as opportunistic microorganisms were isolated during the study. The results show a need for continuous monitoring of bacterial resistance trends.

Keywords: Microbial keratitis; Anti microbial; *Staphylococcus epidermidis*; *Pseudomonas aeruginosa.*

Introduction

Microbial keratitis is an ocular emergency common among contact lens wearers. Incorrect usages and unhygienic maintenance of contact lenses is a leading cause of such eye infections among contact lens wearers. It requires prompt and appropriate management to ensure best visual outcome for the patient. If possible, this means identification of the causative organisms and selection of the best antibiotic to treat the patient. Considering the normal flora of eyes and the virulence of different organisms which are responsible for microbial keratitis, it becomes very difficult for the ophthalmologist to prescribe proper antibiotic because in our health conditions when a patient visit an ophthalmologist, he requires immediate treatment, so usually fortified combined antibiotics are prescribed to provide broad spectrum coverage without the reports of culture and sensitivity.

For medical management of microbial keratitis, treatment with broad spectrum antibiotics may be instituted before pathogen identification and antibiotic susceptibility tests are available (1). Antibiotics are generally effective for treatment of most cases of bacterial ocular infections. However, holes in therapy emerge because of the frequent indiscriminate

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use of antibiotics, which has led to the development of resistance to many commonly used anti microbial (2-4). Hence, periodic susceptibility testing should be carried out as proposed by many researchers to detect the resistance trends (5, 6).

There were more culture positive contact lenses and/or storage cases than culture positive corneal scrapings,hence similar bacteria were isolated from the two sources in only 25% of all cases (7). This demonstrates that bacteria recovery from lenses and storage cases is easier than from cornea; however, it cannot be considered as a reliable guide for directing antibiotic therapy. Therefore, reliance on culture sensitivity data from contact lens and/or storage cases may results in sub-optimal treatment of such conditions. During the present study, we determined the culture sensitivity of the isolates recovered from conjunctiva of contact lens wearer after the removal of contact lenses. Hence, this study could provide information on the efficacy of ocular antibiotics commonly used against bacterial pathogens of keratitis. It is hoped that this information will be helpful in empiric treatment of bacterial keratitis.

Experimental

Study population

The sample population was the Faculty of Pharmacy, University of Karachi undergraduate students wearing contact lenses. The reason for such choice was the accessibility of the subjects. Only students using contact lenses were considered subject volunteers, who had established a routine pattern of lens care. This study was a prospective study and the study period was 12 months from Feb 2005 to Jan 2006.

Isolation and identification procedure

The samples were taken from conjunctiva of right eye of the subjects using sterile cotton swabs. Each swab obtained was inoculated into separate tubes with brain heart infusion (BHI) broth and incubated at 37°C for 24 h. Bacterial isolation was conducted by obtaining inoculums from the incubated BHI broth and gently streaking it on blood agar, MacConkey's agar, Vogel-Johnson's agar, cetrimide agar and nutrient agar. Bacterial culture obtained was identified using Gram's staining, performing biochemical tests (including catalase, coagulase and oxidase) and on the basis of culture diagnosis by growing on selective media. To separate individual cells, streaking of bacterial culture on nutrient agar plate was used. These plates were incubated at 37°C for 12 h. Pure cultures were further confirmed by performing Gram's staining and by studying the morphology of these isolated colonies.

Table 1. Antibiotic discs used during this study.

series number	Name of antibiotic	Amount of antibiotic (in microgram) per disc
1	Amoxycillin	10
2	Cephradine	10
3	Neomycin	30
4	Ciprofloxacin	5
5	Chloramphenicol	30
6	Imipenem	10

Antibiotic sensitivity testing

Each isolate was grown in 5 ml Mueller-Hilton broth at 37°C for 2-8 ho and making a loan on Mueller-Hilton agar. Appropriate antibiotic impregnated discs were placed on the agar surface. Antibiotics used were amoxycillin, cephradine, neomycin, chloramphenicol, imipenem and ciprofloxacin (Table 1). Plates were incubated at 35-37°C for 24 h. Diameter of the zone of inhibition around each disc was measured with a vernier caliper and they were interpreted as sensitive or resistant, according to the zone interpretive standards (8). Isolates of intermediate sensitivity were categorized, as resistant as the number of intermediate isolates was insignificant, when compared to the whole sample.

Results and Discussion

Microbial keratitis is a corneal infection common among contact lens wearer. Isolation and identification of pathogens from contact lenses, as well as conjunctiva, may suggest an appropriate chemotherapy. Hence, for the present study conjunctival swabs from contact lens wearer were screened for the presence of

Table 2. Sensitivity results of different groups isolated from contact lens wearers to amoxycilin, cephradine, neomycin, ciprofloxacin, chloramphenicol and imipenem.

Microorganisms	Number sensitive/Number tested (% Sensitivity)					
	Amoxycillin	Cephradine	Neomycin	Ciprofloxacin	Chloramphenicol	Imipenem
S. epidermidis	34/41 (82.9)	31/41 (75.6)	39/41 (95)	38/41 (92.9)	36/41 (87.8)	41/41 (100)
P. aeruginosa	1/39 (2.6)	3/39 (7.7)	27/39 (69.2)	32/39 (82)	11/39 (28.3)	33/39 (84.4)
S. aureus	6/11 (54.6)	3/11 (27.3)	11/11 (100)	10/11 (100)	9/11 (81.1)	11/11 (100)
Bacillus species	2/7 (28.6)	2/7 (28.6)	5/7 (71.4)	6/7 (85.7)	4/7 (57.2)	7/7 (100)
E. coli	0/2 (0)	0/2 (0)	2/2 (100)	1/2 (50)	1/2 (50)	2/2 (100)

bacteria. After identification we also determined culture sensitivity. Results are depicted in Table 2.

Ocular isolates of *S. epidermidis* (n=41) showed a highly variable pattern of antibiotic sensitivity. 82.9% and 75.6% of isolates were sensitive to amoxycillin and cephradine, respectively, whereas isolates sensitive to neomycin, ciprofloxacin and chloramphenicol were found to be 95%, 92.9% and 87.8%, respectively. It was observed that 92% of *S. epidermidis* had susceptibility to ciprofloxacin (9). Another researcher reported that 11% of *S. epidermidis* strains were ciprofloxacin resistant (10). During the present study, it is a growing evidence for antibiotic resistance among ocular strains of *S. epidermidis* was observed.

Antibiotic susceptibility of *P. aeruginosa* (n=39) was found to be 2.6% to amoxycillin, 7.7% to cephradine, 69.2% to neomycin, 82% to ciprofloxacin, 84.4% to imipenem and 28.3% to chloramphenicol, respectively. In 1999, it was noted that 15.6% cases of *Pseudomonas* karatitis were caused by ciprofloxacin–resistant *Pseudomonas* (4).

Antibiotic susceptibility of ocular strains of *P. aeruginosa* to ciprofloxacin and imipenem observed during the present study is similar to the earlier reports (11, 12).

Staphylococcus aureus (n=11) sensitivity was found to be 54.6% to amoxycillin, 27.3% to cephradine, 100% to neomycin, 100% to ciprofloxacin, 81.1% to chloramphenicol and 100% to imipenem. It was reported that the resistance of *S. aureus* to ciprofloxacin increased by 7% (13). Resistance to chloramphenicol among Gram positive organisms was 10% (14), whereas 75% of Gram positive ocular isolates were sensitive or intermediate sensitive to neomycin, and 70% sensitive or intermediate sensitive to chloramphenicol (15).

Seven isolates of *Bacillus species* analyzed during this study showed 28.6% sensitivity to amoxycillin, 28.6% to cephradine, 71.4% to neomycin, 57.2% to chloramphenicol, 85.7% to ciprofloxacin and 100% to imipenem. It was reported that all isolates of *Bacillus species* were sensitive to gentamicin, tobramycin and ciprofloxacin (16). Two ocular isolates of *E. coli* used during the study showed 0% sensitivity to amoxycillin and cephradine, 50% to ciprofloxacin and chloramphenicol, and 100% to neomycin and imipenem. Previous reports showed that 10% of the Gram positive bacilli from the patients of bacterial keratitis were not sensitive to ciprofloxacin (2). Percentage sensitivity to different antibiotics of *Bacillus species* and *E. coli*, as exhibited in this study, may partly be attributed to the fact that the total number of isolates was too small.

Most of the organisms isolated during this study were resistant to amoxycillin, cephradine, neomycin and chloramphenicol. This study suggests that ciprofloxacin and imipenem are the best choices for treating microbial keratitis. Monotherapy with fluoroquinolones eye drops is a better choice than combined antibiotic therapy, due to the quicker clinical response and less toxicity (17). In addition, fluoroquinolones have good sensitivity to common ocular isolates (18). It is common practice that a patient come to the ophthalmologist when an infection becomes so severe that the ophthalmologist cannot wait for the results of sensitivity tests and has to prescribe a broad-spectrum antibiotic but treatment modification can be made after getting the results of sensitivity test (19). As the above study showed the development of resistance, it is advisable to perform sensitivity test and modify the chemotherapy according to the results of the sensitivity test.

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