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PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIAL PATHOGENS ISOLATED FROM CONJUNCTIVITIS

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ABSTRACT

Antibacterial susceptibility tests against 108 strains belonging to 9 genera, isolated from conjunctivitis, were carried out by disc diffusion method. These comprised *Staphylococcus warnei* (13 strains), *S. intermedius* (10), *S. epidermidis* (10), *S. lugdunensis* (10), *S. simulans* (7), *S. auricularis* (6), *S. schleiferi* (4), *S. aureus* (6), *S. haemolyticus* (2), *S. capitis* (2), *S. saprophyticus* (1), *Micrococcus nishinomyaensis* (5), *M. varians* (4), *M. kristinea* (2), *M. sedentarius* (1), *Streptococcus morbillorum* (4), *S. pyogenes* (1), *Bacillus coagulans* (1), *B. sphaericus* (2), *B. firmus* (4), *Corynebacterium pseudodiphtheriticum* (4), *C. mycetoides* (1), *Pseudomonas aeruginosa* (3), *P. mallei* (1), *Moraxella osleonsis* (2), *Haemophilus aphrophilus* (1), and *Branhamella catarrhalis* (1). In the present study 36.1% isolates were found resistant to ampicillin, 2.7% to chloramphenicol, 8% to clindamycin, 0.9% to gentamicin, 50% to polymyxin B, 14.8% to tetracycline and 1% to vancomycin while all isolates were susceptible to sulfonamides and trimethoprim. It was also observed that 28.7% strains were single-drug-resistant, 7.4% strains were resistant to 2 antibiotics while 0.9% strains were multi-drug-resistant.

Key words: Conjunctivits, antibiotic resistant, multi-drug resistant, chloramphenicol, polymyxin B.

INTRODUCTION

Conjunctivitis is an inflammation of conjunctiva, the mucous membrane that lines the eyelid and covers the white of the eyeball. The most common cause of conjunctivitis is a viral infection. Other causes include bacterial infection and reactions to eye medications (Callahan, 2006).

Bacterial conjunctivitis, being a major cause of ocular morbidity, remains a very important problem for medical practitioners (Petricek *et al.*, 2006; Adegbehingbe and Onipede, 2005). *Staphylococcus aureus* is the most common cause of bacterial conjunctivitis (Modarres *et al.*, 1998). Other bacterial pathogens include *Streptococcus* spp., *Haemophilus influenzae.* (Meurer and Slawson, 2001), *Staphylococcus epidermidis* (Modarres *et al.*, 1998; Khan *et al.*, 2004), *Pseudomonas aeruginosa* (Sun *et al.*, 2002), *Corynebacterium diphtheriae* and *Corynebacterium xerosis* (Miller, 1978), *Moraxella lacunata*, *Acinetobactor* spp., *Neisseria gonorrhoeae, Branhamella catarrhalis* and some anaerobic bacteria. *Escherichia coli, Proteus, Klebsiella* and *Viridans streptococci* have also been recovered in less frequency (Modarres *et al.*, 1998; Berrocal *et al.*, 2001).

In case of bacterial conjunctivitis, broad spectrum antibiotics are commonly administered to hasten recovery and reduce complications (Senaratne and Gilbert, 2005). However, the increasing emergence of antimicrobial resistance and the dissemination among bacterial strains reduce the efficiency of treatment success of many drugs (Sechi *et al.*, 1999; Ates and Erdogrul, 2003; Nair and Chanda, 2005). Muti-drug-resistant strains further complicate the therapy of infections (Callaghan *et al.*, 1997). The aim of this study was to evaluate the antibiotic sensitivity of the bacterial pathogens of conjunctivitis (Sahar, 2005).

MATERIALS AND METHODS

Media

Mueller-Hinton agar (MHA) (Merck) was used as antibiotic susceptibility test medium and Mueller-Hinton broth (MHB) (Merck) was used for preparation of inoculum.

Preparation of plates

The plates of 100 mm diameter were used for antibiotic susceptibility test. MHA (20 ml) was poured into sterile petri plates to get a depth of 4-6 mm. All the plates were incubated for 24 hours to check sterility.

Antibiotic discs

Different antibiotic discs (Table 1) were used for antibiotic susceptibility test.

Preparation of 0.5 McFarland Nephelometer Standard

McFarland tube number 0.5 was prepared by mixing 0.5 ml 1.175% barium chloride solution and 99.5 ml 1% sulphuric acid solution.

Inoculum

Four to five colonies from pure growth of organisms were transferred to 5 ml MHB. The broth was incubated at 37^{o}C for 18 - 24 hours. The turbidity of the culture was compared to 0.5 McFarland turbidity standard. The standardized inoculum was inoculated within 15 – 20 minutes.

Inoculation of medium

A sterile cotton swab was immersed into the standardized inoculum. Excess broth was drained off by pressing and rotating the swab against the wall of tube. It was streaked evenly in three directions on the surface of agar plate. A final circular motion was made around the agar rim with the cotton swab. These plates were allowed to dry for 3–5 minutes.

Disc placement

Antibiotics discs were placed on the surface of inoculated plates by using a sterile forcep. After placement the discs were pressed gently to the agar surface. The inoculated plates with discs were incubated at 35–37^{o}C for 18–24 hours.

Interpretation

Inhibition zone diameters were measured in mm and the susceptibility or resistance of the organisms were interpreted on the basis of criteria mentioned in Table 1.

Table 1. Criteria for the interpretation of antibiotic resistance/susceptibility.

Antibiotics	**potency**	**Inhibition zone diameter in mm**		
	(μg)	**Resistant**	**Intermediate**	**Susceptible**
Ampicillin	10			
Gram negative		≤ 13	14 – 16	≥ 17
Staphylococci		≤ 28	---	≥ 29
Haemophilus		≤ 21	22 – 24	≥ 25
Chloramphenicol	30	≤ 12	13 – 17	≥ 18
Haemophilus		≤ 25	26 – 28	≥ 29
Clindamycin	02	≤ 14	15 – 20	≥ 21
Gentamicin	10	≤ 12	13 – 14	≥ 15
Polymyxin B	300	≤ 8	9 – 11	≥ 12
Tetracyclin	30	≤ 14	15 – 18	≥ 19
Haemophilus		≤ 25	26 – 28	≥ 29
Vancomycin	30	≤ 14	15 – 16	≥ 17
Sulfonamides and trimethoprim	24	≤ 10	11 – 15	≥ 16

RESULTS AND DISCUSSION

Antibiotic resistance is one of the major public health concern, **t**herefore, The selection of specific antimicrobial therapy should be based on the findings of laboratory studies (Coad *et al.,* 1984; Modarres *et al.,* 1998). Acute conjunctivitis is frequently a self-limiting condition, but the use of antibiotics is associated with significantly improved rates of clinical and microbiological remission (Sheikh and Hurwitz, 2006). The high sensitivity of the isolates isolated from bacterial conjunctivitis to ciprofloxacin, chloramphenicol and gentamicin supports the appropriateness of using these drugs as first line drugs in the management of bacterial conjunctivitis. (Adegbehingbe and Onipede, 2005).

The inappropriate use of antibiotics greatly accelerates the emergence of antibiotic- resistance among bacteria (Chapin *et al.,* 2005; Saeed *et al.,* 2005).

In the present study, 108 strains belonging to 9 different genera viz., *Staphylococcus* (*S. warnei, S. intermedius, S. epidermidis, S. lugdunensis, S. simulans, S. auricularis*, *S. schleiferi, S. aureus, S. haemolyticus, S. capitis, S.*

saprophyticus), *Micrococcus* (*M. nishinomyaensis M. varians, M. kristinea, M. sedentarius*), *Streptococcus* (*S. morbillorum, S. pyogenes*), *Bacillus* (*B. coagulans, B. sphaericus, B. firmus*), *Corynebacterium* (*C. pseudodiphtheriticum, C. mycetoides*), *Pseudomonas* (*P. aeruginosa, P. mallei*), *Moraxella osleonsis, Haemophilus aphrophilus*, and *Branhamella catarrhalis* were used for the evaluation of antibiotic resistance. Most of these species are the normal flora of conjunctiva (Fleisig and Efron, 1992; Sechi *et al.,* 1999; Berry *et al.,* 2002) and however some of these have also been reported to be involved in conjunctivitis (Willcox *et al.,* 1998; Chung *et al.,* 2000; Berrocal *et al.,* 2001; Fukuda *et al.,* 2002).

Table 2. Antibiotic resistance pattern of bacteria.

Organisms	**No. of**	**No. of isolates resistant to antibiotics**							
	Isolates	**AM**	**C**	**CC**	**GM**	**PB**	**TE**	**VA**	**SXT**
S.warneri	13	8	1	0	0	-	9	0	0
S.intermedius	10	7	0	0	0	-	0	0	0
S.epidermidis	10	6	2	0	0	-	0	0	0
S.lugdunensis	10	5	0	0	0	-	0	0	0
S.simulans	07	5	0	4	0	-	0	0	0
S.auricularis	06	0	0	0	0	-	0	0	0
S.schleiferi	04	2	0	0	0	-	2	0	0
S.aureus	06	0	0	0	0	-	0	0	0
S.haemolyticus	02	1	0	0	0	-	1	0	0
S.capitis	02	0	0	0	0	-	0	0	0
S.saprophyticus	01	1	0	0	0	-	0	0	0
M.nishinomyaensis	05	0	0	0	0	-	0	0	0
M.varians	04	0	0	0	0	-	0	0	0
M.kristinae	02	0	0	1	0	-	0	1	0
M.sedentarius	01	0	0	0	0	-	1	0	0
S.morbillorum	04	2	0	0	0	-	0	0	0
S.pyogenes	01	1	0	0	0	-	0	0	0
B.coagulans	01	0	0	0	0	-	0	0	0
B.sphaericus	02	0	0	1	0	-	0	0	0
B.firmus	04	0	0	2	0	-	0	0	0
C.pseudodiphtheriticum	04	0	0	0	0	-	0	0	0
C.mycetoides	01	0	0	0	0	-	0	0	0
P.aeruginosa	03	0	0	-	1	1	1	-	0
P.mallei	01	1	0	-	0	1	1	-	0
M.osloensis	02	0	0	-	0	2	1	-	0
H.aphrophilus	01	0	0	-	0	0	0	-	0
B.catarrhalis	01	0	0	-	0	0	0	-	0
Total	**108**	**39**	**3**	**8**	**1**	**4**	**16**	**1**	**0**
Percentage (%)	**100**	**36.1**	**2.7**	**8.0**	**0.9**	**50**	**14.8**	**0.9**	**0**

Key: AM = Ampicillin, C = Chloramphenicol, CC = Clindamycin, GM = Gentamicin, PB = Polymyxin B, TE = Tetracyclin, VA = Vancomycin, S = Streptomycin, - = Not done

The sensitivity to antibiotics is varied greatly among the bacteria. It was found that 36.1% (39/108) strains were resistant to ampicillin, 2.7% (3/108) to chloramphenicol, 8% (8/100) to clindamycin, 0.9% (1/108) to gentamicin, 50% (4/8) to polymyxin B, 14.8% (16/108) to tetracycline, and 1% (1/100) were resistant to vancomycin while all tested isolates were found susceptible to sulfomamide and trimethoprim (Table 2). Chloramphenicol is a potent broad-spectrum antibiotic and is still a widely prescribed for ocular infections (Bron *et al.,* 1991; Rose *et al.,* 2005; Everitt *et al.,* 2006). In contrast in another study carried out by Locatelli *et al.* (2003) Gram negative bacteria presented a high degree of resistance to chloramphenicol.

In the present study, single-drug-resistance was more common than multi-drug-resistance. It was observed that 28.7% (31/108) strains were single drug resistant and 7.4% (8/108) strains were found resistant to 2 antibiotics while only 0.9% (1/108) were resistant to 3 antibiotics (Table 3). These results are in fair correlation with a previous study carried out by Modarres *et al.* (1998), who investigated that many of the bacteria were sensitive to antibiotics and single-drug-resistant strains were more common as compared to multi-drug-resistant strains.

Table 3. Emergence of multi-drug resistance among bacteria.

Organisms	No. of Isolates	No. of isolates resistant to no. of antibiotics						
		None	1	2	3	4	5	6	7
S.warneri	13	11	2	0	0	0	0	0	0
S.intermedius	10	6	4	0	0	0	0	0	0
S.epidermidis	10	2	8	0	0	0	0	0	0
S.lugdunensis	10	7	3	0	0	0	0	0	0
S.simulans	07	4	2	1	0	0	0	0	0
S.auricularis	06	6	0	0	0	0	0	0	0
S.schleiferi	04	2	1	1	0	0	0	0	0
S.aureus	06	6	0	0	0	0	0	0	0
S.haemolyticus	02	1	0	1	0	0	0	0	0
S.capitis	02	2	0	0	0	0	0	0	0
S.saprophyticus	01	0	1	0	0	0	0	0	0
M.nishinomyaensis	05	5	0	0	0	0	0	0	0
M.varians	04	4	0	0	0	0	0	0	0
M.kristinae	02	0	2	0	0	0	0	0	0
M.sedentarius	01	0	1	0	0	0	0	0	0
S.morbillorum	04	3	1	0	0	0	0	0	0
S.pyogenes	01	0	1	0	0	0	0	0	0
B.coagulans	01	0	1	0	0	0	0	0	0
B.sphaericus	02	1	1	0	0	0	0	0	0
B.firmus	04	2	1	1	0	0	0	0	0
C.pseudodiphtheriticum	04	4	0	0	0	0	0	0	0
C.mycetoides	01	1	0	0	0	0	0	0	0
P.aeruginosa	03	0	1	1	1	0	0	0	0
P.mallei	01	0	0	1	0	0	0	0	0
M.osloensis	02	0	0	2	0	0	0	0	0
H.aphrophilus	01	1	1	0	0	0	0	0	0
B.catarrhalis	01	1	0	0	0	0	0	0	0
Total	108	69	31	8	1	0	0	0	0

REFERENCES

Adegbehingbe, B.O., and A.O. Onipede (2005). Conjunctivitis as seen in Ile-Ife. *Nigerian Journal of Opthalmology*, 13(1): 21-26.

Ates, D.A. and O.T. Erdogrul (2003). Antimicrobial activities of various medicinal and commercial plant extracts. *Turk J. Biol*., 27: 157-162.

Berrocal, A.M., I.U. Scott, DS. Miller, and H.W. Flynn (2001). Endophthalmitis caused by *Moraxella* species. *AJO*, 132: 788-790.

Berry, M., A. Harris, R. Lumb and K. Pawell (2002). Commensal ocular bacteria degrade mucin. *Br. J. Ophthalmol*, 86: 1412-1416.

Bron, A.J., G. Laber, S.N. Rizk, H. Baig, A.R. Elkington, G.R. Kirkby, C. Neoh, A. Harden and T. Leong (1991). Ofloxacin compared with chloromphenicol in the management of external ocular infections. *British Journal of Ophthalmology*, 75(675-679.

Callaghan, R.J., M.C. Callegan, J.M. Moreau, L.C. Green, T.J. Foster, O.M. Hartford, L.S. Engel and J.M. Hill (1997). Specific roles of alpha-toxin and beta-toxin during *Staphylococcus aureus* corneal infection. *IAI*, 65: 1571-1578.

Callahan, D. (2006). Conjunctivitis. http://www.djo.harvard.edu/site.php?url=/patients /pi/410.

Chapin, A., A. Rule, K. Gibson, T. Buckley and K. Schwab (2005). Airborne multi-drug-resistant bacteria isolated from a concentrated swine feeding operation. *Environmental Health Perspectives*, 113: 137-142.

Chung, C.N., C. Fellow, and E.J. Cohen (2000). Clinical evidence of eye disorders: bacterial conjunctivitis. *WJM*, 173: 202-205.

Coad, C.T., M.S. Osato and K.P. Wilhelmus (1984). Bacterial contamination of eyedrop dispensers. *American Journal of ophthalmology*, 98: 548-551.

Everitt, H.A., P.S. Little, and W.F. Smith (2006). A randomized controlled trail of management strategies for acute infective conjunctivitis in general practice. *BMJ*, 333: 321-323.

Fleisig S.M.J. and N. Efron (1992). Microbial flora in eyes of current and former contact lens wearers. *JCM*, 30: 1156-1161.

Fukuda, M., H. Ohashi, C. Matsumato, and M. Slimomuray (2002). Methicillin-resistant *Staphylococcus aureus* and methicillin-resistanr coagulase-negative staphylococcus ocular surface infections: efficacy of chloramphenicol eye drop. *Cornea*, 21(7S): S86-89.

Khan, A.J¸ M. Shahwani, M. Saleem, and B. Khan (2004). Aerobic bacteriology of normal conjunctival flora. *Pakiatan J. Ophthalmal*., 20: 91-95.

Leibowitz, H.M. (2000). The red eye. *New England Journal of Medicine*, 343: 345-351.

Locatelli, C.I., S. Kwitko, and A.B. Simonetti (2003). Conjunctival endogenous microbiota in patients submitted to cataract surgery. *Braz. J. Microbiol*, 34(3): 1517-1525.

Meurer, L.N., and J.G. Slawson (2001). Are topical antibiotics effective in treating bacterial conjunctivitis? Brief Article- Statistical data included. *Journal of Family Practice*, 13(1): 46-48.

Miller, S.J.H. (1978). PARSON's disease of the eye. 16th edition. ELBS and Churchill Livingstone. pp. 151-430.

Modarres, S., A. Lasheii and N. Oskoii (1998). Bacterial etiologic agents of ocular infection in children in the Islamic Republic of Iran. *Eastern Mediterranean Health Journal*, 4(1): 44-49.

Nair, R., and S. Chanda (2005). Anticandidal activity of *Punica granatum* exhibited in different solvents. *Pharmaceutical Biology*, 43(1): 21-25.

Petricek, I., M. Prost, and A. Popova (2006). The differential diagnosis of red eye: survey of medical practitioners from Eastern Europe and the Middle East. *Opthalmologica*, 220(4): 229-237.

Rose, P.W., A. Harnden, A.B. Brueggemann, R. Perera, A. Sheikh, and D. Crook (2005). Chloramphenicol treatment for acute infective conjunctivitis in children primary care: a randomized double-blind placebo-controlled trial. *Lancet*, 366: 37-43.

Saeed, S., N. Masood, and P. Tariq (2005). A study on prevalence of antibiotic resistant *Bacillus* species. *Int. J. Biol. Biotech*., 2(4): 909-912.

Sahar, A. (2005). Bacterial spectrum of conjunctiva of normal and diseased individuals. M.Sc. Thesis. Department of Micribiology, University of Karachi, Karachi.

Sechi, L.A., A. Pinna, S. Pusceddu, G. Fadda, F. Carta and S. Zanetti (1999). Molecular characterization and antibiotic susceptibilities of ocular isolates of *Staphylococcus epidermidis. JCM*, 37: 3031-3033.

Sheikh, A and B. Hurwitz (2006). Antibiotics versus placebo for acute bacterial conjunctivitis. *Cochrane Database Syst Rev*, 2: CD001211..

Sun, X. G., Z.Q. Wang, L. Chen, S.Y. Luo, X.Y. Jin, and W.H. Zhang (2002). Etiologic analysis on bacterial ocular disease in northern China (1989-1998). *Chin. Med. J*., 6: 933-935.

Willcox, M.D.P., A. Thakur, and B.A. Holden (1998). Some potential pathogenic traits of Gram-negative bacteria isolated during ocular inflammation and infections. *Clinical and Experimental Optometry*, 81: 56-62.

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