

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 osloensis* (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: Conjunctivitis, 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*, *Acinetobacter* 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 Erdogru, 2003; Nair and Chanda, 2005). Multi-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°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°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 (µg)	Inhibition zone diameter in mm		
		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, therefore, 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 osloensis*, *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
<i>S.warneri</i>	13	8	1	0	0	-	9	0	0
<i>S.intermedius</i>	10	7	0	0	0	-	0	0	0
<i>S.epidermidis</i>	10	6	2	0	0	-	0	0	0
<i>S.lugdunensis</i>	10	5	0	0	0	-	0	0	0
<i>S.simulans</i>	07	5	0	4	0	-	0	0	0
<i>S.auricularis</i>	06	0	0	0	0	-	0	0	0
<i>S.schleiferi</i>	04	2	0	0	0	-	2	0	0
<i>S.aureus</i>	06	0	0	0	0	-	0	0	0
<i>S.haemolyticus</i>	02	1	0	0	0	-	1	0	0
<i>S.capitis</i>	02	0	0	0	0	-	0	0	0
<i>S.saprophyticus</i>	01	1	0	0	0	-	0	0	0
<i>M.nishinomyaensis</i>	05	0	0	0	0	-	0	0	0
<i>M.varians</i>	04	0	0	0	0	-	0	0	0
<i>M.kristinae</i>	02	0	0	1	0	-	0	1	0
<i>M.sedentarius</i>	01	0	0	0	0	-	1	0	0
<i>S.morbillorum</i>	04	2	0	0	0	-	0	0	0
<i>S.pyogenes</i>	01	1	0	0	0	-	0	0	0
<i>B.coagulans</i>	01	0	0	0	0	-	0	0	0
<i>B.sphaericus</i>	02	0	0	1	0	-	0	0	0
<i>B.firmus</i>	04	0	0	2	0	-	0	0	0
<i>C.pseudodiphtheriticum</i>	04	0	0	0	0	-	0	0	0
<i>C.mycetoides</i>	01	0	0	0	0	-	0	0	0
<i>P.aeruginosa</i>	03	0	0	-	1	1	1	-	0
<i>P.mallei</i>	01	1	0	-	0	1	1	-	0
<i>M.osloensis</i>	02	0	0	-	0	2	1	-	0
<i>H.aphrophilus</i>	01	0	0	-	0	0	0	-	0
<i>B.catarrhalis</i>	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 sulfonamide 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		
<i>S.warneri</i>	13	11	2	0	0	0	0	0	0	
<i>S.intermedius</i>	10	6	4	0	0	0	0	0	0	
<i>S.epidermidis</i>	10	2	8	0	0	0	0	0	0	
<i>S.lugdunensis</i>	10	7	3	0	0	0	0	0	0	
<i>S.simulans</i>	07	4	2	1	0	0	0	0	0	
<i>S.auricularis</i>	06	6	0	0	0	0	0	0	0	
<i>S.schleiferi</i>	04	2	1	1	0	0	0	0	0	
<i>S.aureus</i>	06	6	0	0	0	0	0	0	0	
<i>S.haemolyticus</i>	02	1	0	1	0	0	0	0	0	
<i>S.capitis</i>	02	2	0	0	0	0	0	0	0	
<i>S.saprophyticus</i>	01	0	1	0	0	0	0	0	0	
<i>M.nishinomyaensis</i>	05	5	0	0	0	0	0	0	0	
<i>M.varians</i>	04	4	0	0	0	0	0	0	0	
<i>M.kristinae</i>	02	0	2	0	0	0	0	0	0	
<i>M.sedentarius</i>	01	0	1	0	0	0	0	0	0	
<i>S.morbilloorum</i>	04	3	1	0	0	0	0	0	0	
<i>S.pyogenes</i>	01	0	1	0	0	0	0	0	0	
<i>B.coagulans</i>	01	0	1	0	0	0	0	0	0	
<i>B.sphaericus</i>	02	1	1	0	0	0	0	0	0	
<i>B.firmus</i>	04	2	1	1	0	0	0	0	0	
<i>C.pseudodiphtheriticum</i>	04	4	0	0	0	0	0	0	0	
<i>C.mycetoides</i>	01	1	0	0	0	0	0	0	0	
<i>P.aeruginosa</i>	03	0	1	1	1	0	0	0	0	
<i>P.mallei</i>	01	0	0	1	0	0	0	0	0	
<i>M.osloensis</i>	02	0	0	2	0	0	0	0	0	
<i>H.aphrophilus</i>	01	1	1	0	0	0	0	0	0	
<i>B.catarrhalis</i>	01	1	0	0	0	0	0	0	0	
Total	108	69	31	8	1	0	0	0	0	

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(Accepted for publication October 2006)