

## Antimicrobial susceptibility of bacteria isolated from children infected eyes in Benha University hospital

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### ABSTRACT

The aim of current study is to characterize bacteria isolated from eye infections and evaluate the effectiveness of some antimicrobials found in eye drops in the Egyptian Market. Clinical bacterial isolates were recovered from patients having conjunctivitis from Benha University Hospital and identified using standard microbiological techniques. Antimicrobial susceptibility of these isolates against various antimicrobials was tested by the disc diffusion method.

A total of eighty one bacterial isolates were identified in this study, 30 Gram positive isolates and 51 Gram negative isolates. The major bacterial isolates were *Pseudomonas aeruginosa* (37%) and *Staphylococcus spp.* (37%). All the isolates were susceptible to fluoroquinolones, and almost all of them were susceptible to aminoglycoside antibiotics. While 75 isolates were susceptible to tetracycline and 71 isolates were susceptible to chloramphenicol. High resistance rates were observed for both bacitracin (45.7%) and fusidic acid (60.4%). Seven isolates were multidrug resistant (MDR).

This study demonstrated that isolates from infected eye were highly sensitive to fluoroquinolones and aminoglycosides. This study recommends that antimicrobial susceptibility should be performed for bacteria isolated from patients' eyes before antimicrobial therapy to avoid emergence of MDR isolates

**Key words:** eye infections, *Staphylococcus spp.*, *Pseudomonas*, antimicrobial resistance,

### INTRODUCTION

The human's eyes are protected by many factors that prevent ocular infections (Kanski and Bowling, 2011). The microflora of conjunctival sac has been investigated all over the world; mostly it is sterile (Capriotti *et al.*, 2009). For non-sterile but healthy conjunctival sacs, the most commonly isolated organism was *Staphylococcus spp*, mostly *S. epidermidis* and *S. aureus* (Sharma *et al.*, 2013). However, under certain circumstances, microorganisms gain access to the eye and cause a variety of infections (Fleiszig, 2006). The most common bacteria that could cause eye infections are *Staphylococcus*

*aureus* followed by *Sterptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Hemophilus influenza* and *Klebsiella species* (Bharathi *et al.*, 2010).

Eye infections could be treated topically using aminoglycosides, quinolones, fusidic acid, chloramphenicol, polypeptides, tetracycline and sulphacetamide (Mohamed and Kreling, 2016). Resistance and sensitivity profiles based on *in vitro* testing may not reflect true clinical resistance and response to an antibiotic because of host related factors. Tetracycline and chloramphenicol were found to be the most effective drugs, especially when used topically (Nakhla *et*

*al.*, 1970; Seal *et al.*, 1982). Recently, there is a slight decrease in susceptibility of bacteria isolated from infected eyes to ciprofloxacin and ofloxacin (Bharathi, 2010; Chhablani, 2013). Also Mshangila *et al.* (2013) reported increased resistance to tobramycin, while Muluye *et al.* (2014) showed that most of bacterial isolates from eye infection were resistant to tetracycline.

The aim of current study is to determine the types of bacterial isolates causing eye infections in children living in Benha, Qalyubia, Egypt. In addition the susceptibility of bacteria isolated from eye infections to various antibiotics was determined for better choice of therapeutic options.

## MATERIALS and METHODS

### Bacteria isolation and identification

A total of one hundred and sixteen clinical specimens were collected from eyes of children diagnosed as having conjunctivitis and attending the Ophthalmic Department of Benha University Hospital, Egypt. All specimens were taken by moistened sterile swab. The swabs were cultured on chocolate agar, blood agar, MacConkey agar and incubated for 24 hours at 37°C. Isolated bacteria were characterized using Gram staining and biochemical reactions (Collee *et al.*, 1996).

### Antibiotic susceptibility test

Bacterial isolates were tested for their susceptibility *in vitro* against antimicrobial agents by disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2013). The antibiotics discs were purchased from Oxoid (Hampshire, England) and included gatifloxacin (GAT, 5µg), ofloxacin (OFX, 5µg), neomycin (N, 30µg), tobramycin (TOB, 10µg), bacitracin (B, 10µg), tetracycline (TE, 30µg), ciprofloxacin (CIP, 5µg), levofloxacin (LEV, 5µg), gentamycin (CN, 10µg),

fucidic acid (FD, 10µg) and chloramphenicol (C, 30µg). Diameters of inhibition zones were measured and results were interpreted according to CLSI (2013).

## RESULTS

### Identification of bacterial isolates:

A total of 81 bacterial isolates were recovered from clinical samples. Among the 81 isolates, 30 isolates (37%) were found to be Gram positive bacteria from which 20 isolates (24.7%) were identified as *S. aureus*, 8 isolates (9.9%) as *S. epidermidis* and 2 isolates (2.46%) as *S. saprophyticus*. The 51 Gram negative bacterial isolates include 30 isolates identified as *P. aeruginosa* (37%), 9 isolates identified as *K. pneumonia* (11.1%), 6 isolates as *Moraxella catarrhalis* (7.4%) and 6 isolates as *Moraxella lacunata* (7.4%) (Table 1).

### Antibiotic susceptibility test (AST):

All isolates were susceptible to fluoroquinolones (Ciprofloxacin, Ofloxacin, Levofloxacin and Gatifloxacin). Almost all isolates were susceptible to aminoglycosides with 78 isolates being susceptible to gentamycin, 76 to tobramycin and 74 to neomycin. While 75 isolates were susceptible to tetracycline and 71 isolates were susceptible to chloramphenicol. Marked resistance was observed to bacitracin and fusidic acid with 37 and 49 isolates being resistant, respectively

Our results show that all *S. aureus* were sensitive to fluoroquinolones, tobramycin and gentamycin, but show high resistance to fusidic acid (70 %). All *S. epidermidis* were sensitive to fluoroquinolones, chloramphenicol, tobramycin, gentamycin and tetracycline, but showed high resistance to fusidic acid (62.5%). While *S. saprophyticus* isolates showed high resistance to both fusidic acid and bacitracin (50%) (Table 2).

**Table 1:** The distribution of specimens and type of bacterial isolates

<b>Specimens</b>	<b>No.</b>	<b>%</b>
No of specimens	116	
Negative specimens*	49	42
Positive specimens	67	58
Multi-infection specimens**	14	20.9
Uni-infection specimens***	53	79.1
<b>Microorganisms</b>		
<i>S. aureus</i>	20	24.6
<i>S. epidermidis</i>	8	9.9
<i>S. saprophyticus</i>	2	2.5
<i>P. aeruginosa</i>	30	37
<i>Klebsiella spp.</i>	9	11.1
<i>M. lacunata</i>	6	7.4
<i>M. catarrhalis</i>	6	7.4
<b>Total isolates</b>	<b>81</b>	<b>100</b>

\*Specimens showing no bacterial growth

\*\*Specimens showing growth of more than one species of bacteria

\*\*\* Specimens showing growth of one species of bacteria

**Table 2:** Pattern of resistance of Gram positive isolates to tested antibiotics (%)

Antibiotic	The resistance percentage of			Total
	<i>S. aureus</i> n = 20	<i>S. epidermidis</i> n=8	<i>S. saprophyticus</i> n = 2	n=30 (%)
<b>Ciprofloxacin</b>	0	0	0	0
<b>Ofloxacin</b>	0	0	0	0
<b>Levofloxacin</b>	0	0	0	0
<b>Gatifloxacin</b>	0	0	0	0
<b>Tobramycin</b>	0	0	0	0
<b>Gentamycin</b>	0	0	0	0
<b>Neomycin</b>	10	25	0	4 (13.3)
<b>Tetracycline</b>	10	0	0	2 (6.7)
<b>Chloramphenicol</b>	10	0	0	2 (6.7)
<b>Bacitracin</b>	10	25	50	5 (16.7)
<b>Fucidic acid</b>	70	62.5	50	20 (67)

NO. of isolates (n)

For Gram negative isolates, *P. aeruginosa* showed moderate resistance to chloramphenicol (26.7%) and relatively high resistance fusidic acid (46.7%) and bacitracin (50%). All *K. pneumonia* isolates

were resistant to fusidic acid, and were highly resistance to bacitracin (77.8%). *M. catarrhalis* isolates showed high resistance to bacitracin (66.7 %). All *M. Lacunata* isolates were resistant to bacitracin, while

66.7% were resistant to fucidic acid and (Table 3). While *M. catarrhalis* showed high resistance to bacitracin (66.7%) and

intermediate resistance to fucidic acid, tetracycline and aminoglycosides. Seven isolates were multidrug resistant (MDR).

**Table 3:** Pattern of resistance of Gram negative isolates to tested antibiotics (%)

Antibiotic	The resistance percentage of				Total n = 51 (%)
	<i>P. aeruginosa</i> n = 30	<i>K. pneumonia</i> n = 9	<i>M. catarrhalis</i> n = 6	<i>M. lacunata</i> n = 6	
Ciprofloxacin	0	0	0	0	0
Ofloxacin	0	0	0	0	0
Levofloxacin	0	0	0	0	0
Gatifloxacin	0	0	0	0	0
Tobramycin	3.3	22.2	33.3	0	5 (9.8)
Gentamycin	3.3	0	33.3	0	3 (5.9)
Neomycin	3.3	0	33.3	0	3 (5.9)
Tetracycline	0	22.2	33.3	0	4 (7.8)
Chloramphenicol	26.7	0	0	0	8 (15.6)
Bacitracin	50	77.8	66.7	100	32 (62.7)
Fucidic acid	46.7	100	33.3	66.7	29 (56.9)

NO. of isolates (n)

## DISCUSSION

The human eye is protected by a number of natural defence mechanisms that protect it from infections (Kanski and Bowling, 2011). Many ocular infections are caused by the use of soft contact lenses (Hou, 2012).

In the present study *P. aeruginosa* was the most isolated organism from eye infection. It was recovered at a frequency of 37 %, followed by *S. aureus* (24.6%), *Klebsiella* (11.1%), *S. epidermidis* (9.9%), *M. lacunata* (7.4%), *M. Catarrhalis* (7.4%) and *S. saprophyticus* (2.5%). Some previous studies recovered *P. aeruginosa* at lower frequencies ranging from 1.25-19% (Quraishy and Ali, 1981; Bharathi, 2010; Hou, 2012; Rahman, 2013). Geographical variability in *P. aeruginosa* acquisition suggested that there are factors such as climate associated with environmental *P. aeruginosa* proliferation and/or affecting

individual susceptibility or exposure to *P. aeruginosa* (Psoter et al., 2014). This explains why our result was higher than others.

In this study *S. aureus* was recovered at a frequency of 24.6%, this was lower than the 32.3% reported by Shrief et al. (1976), and quite similar to results of Shayegani et al. (1982), Anagaw (2011) and Rahman (2013) who recovered *S. aureus* at frequency of 22%, 21.1% and 23.6%, respectively.

Regarding resistance profile, 10 % of *S. aureus* isolates were resistant to bacitracin. Previous studies showed similar resistance to bacitracin (Burns, 1963; Nicholas and Goolden, 1966) ranging from 6% - 20%. All *S. aureus* isolates were susceptible to fluroquinolones including gatifloxacin which is quite similar to what was reported by Bharathi et al. (2010) who showed that Gram positive isolates were 1%

resistant to moxifloxacin and 6.14% resistant to gatifloxacin.

In the current study, *P. aeruginosa* showed high resistance to bacitracin (50 %) and fucidic acid (46.7%). While none of isolated *P. aeruginosa* were resistant to fluroquinolones. similarly **Mehrnejad et al. (2011)** showed that *P. aeruginosa* has no resistance to ofloxacin. while **Bharathi et al. (2010)** showed that *P. aeruginosa* was 8% resistant to gatifloxacin and 11.3% resistant to ofloxacin.

This study showed that 26.7 % of isolated *P. aeruginosa* were resistant to chloramphenicol, this result is comparable with the result of **Seal et al. (1982)** who showed that 15% of isolates were resistant to chloramphenicol. While **Nicholas and Goolden (1966)** showed that it was 70% resistant to chloramphenicol. **Table 3** also showed that none of isolated *P. aeruginosa* were resistant to tetracycline, this result was different from **Seal et al. (1982)** who showed that 15% of *P. aeruginosa* was resistant to tetracycline. While **Nicholas and Goolden (1966)** showed high resistance rate where 70% of isolates was resistant to tetracycline.

Only 3.3 % of *P. aeruginosa* were resistant gentamycin, neomycin and tobramycin. **Nicholas and Goolden (1966) and Seal et al. (1982)** showed that less than 10% of *P. aeruginosa* was resistant to neomycin and gentamycin. Also **Mehrnejad et al. (2011)** showed that 3.5% were resistant to gentamycin. All of *Pseudomonas* isolates were susceptible to ciprofoxacin. This is different from results reported by **Bharathi et al. (2010)** who showed that 13.4% were resistant to ciprofoxacin.

In conclusion, this study found that fluroquinolones are the most effective antimicrobial agents for treatment of eye infections in children. Improper use of antibiotics can lead to emergence of MDR

strains. So choice of antibiotics for treatment of eye infections in children should depend on the antimicrobial resistance profile to avoid therapy failure.

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## مقاومة البكتريا المعزولة من عدوى العيون في الاطفال للمضادات الميكروبية

### غادة شاكر- أميرة الجنائني- عبير ابوالعزم- هبة الداش

استهدفت الدراسة الحالية إلي التعرف علي البكتريا المساهمة في التهاب الملتحمة و التحقيق في المقاومة المتعددة للمضادات الميكروبية الموجودة في قطرات العيون الموجودة في السوق المصري بين البكتيريا المعزولة من عيون مرضي مصابين بالتهاب في الملتحمة.

تم اجراء هذه الدراسة علي 116 عينة منالاطفال الذين تم تشخيصهم بالتهاب في الملتحمة في قسم العيون بمستشفى الجامعة و المستشفى التعليمي و مستشفى الرمذ بينها (عينات سريرية) خلال الفترة من ديسمبر 2013 إلي يناير 2015.

تم التعرف علي 81 عزلة ووجد أنها عبارة عن ستافيلوكوكس أوريس بنسبة 24.7% و ستافيلوكوكس ابيدريميدس بنسبة 9.9% و ستافيلوكوكس سابروفيتكس بنسبة 2.46%. كما تم عزل بكتيريا سودوموناس ايروجينوزا بنسبة 37% و كليبيسيلا نيومونيا بنسبة 11.1% و موراكسيلا كاتاراليز 7.4% و موراكسيلا لاكيوناتا 7.4%.

تم اختبار حساسية البكتريا المعزولة للمضادات الحيوية المختلفة الموجودة في قطرات العيون الموجودة في السوق المصري. أظهرت ستافيلوكوكس اورييس 100% حساسية للتوبراميسين و الجينتاميسين و 91% حساسية للكلورامفينيكول و الجاتيفلوكساسين و أوفلوكساسين و الليفوفلوكساسين و السيبروفلوكساسين و النيومايسين و التيتراسيكلين كما أظهرت مقاومة بسيطة بنسبة 18% للباسيتراسين و مقاومة شديدة لحمض الفوسيديك بنسبة 63%. بينما عزلات الموراكسيلا أظهرت حساسية بنسبة 100% لكل من الأوفلوكساسين و الليفوفلوكساسين و الجاتيفلوكساسين و السيبروفلوكساسين. علي الجانب الآخر أظهرت عزلات السيدوموناس ايروجينوزا حساسية بنسبة 100% لكل من الأوفلوكساسين السيبروفلوكساسين.

و من هذه الدراسة وجد أن الفلوروكينولونز هي أفضل مجموعة من المضادات الميكروبية الي يمكن أن تستخدم لعلاج التهابات العيون الناتجة عن العدوي البكتيرية.