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# Determination of Susceptibility of Causal Agents of Bacterial Conjunctivitis to Antibiotics: Federal Medical Center, Imo State, Nigeria as Case Study

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**ABSTRACT**

The prevalence and sensitivity pattern of common bacterial isolates from conjunctival specimen were investigated. Standard methods were adopted in the collection of samples from neonates, men and women and screening of the isolates. *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus aegyptus* were the predominant pathogens cultured, representing 28.12%, 23.08% and 19.23% respectively. *Escherichia coli*, *Neisseria* and *Corynebacterium* species were also isolated in different proportions. Some of the isolates are resistant to the antibiotics tested. *Staphylococcus aureus* for instance recorded 100% resistance to nitrofurantoin, ceporex, ampicillin, nalidixic acid, tetracycline and cotrimoxazole. *Streptococcus pneumoniae*, one of the most frequent isolates was 100 % resistance to tetracycline and cotrimoxazole. All the isolates were resistant to chloramphenicol. Dust in the environment, infection from the birth canals of mothers, use of eye protective devices such as eye patches, contaminated hands and body surfaces as well as inadequate surgical procedures had been reported as potential sources of causative agents of conjunctivitis. The prevalence of multiple drug resistant conjunctival bacteria had been documented. Multiple antibiotic resistances are increasingly common in *Staphylococcus aureus* and *Neisseria gonorrhea*, which are major cause of nosocomial infection in hospital environment. Genetics of causal agents of conjunctivitis has been proved to be of great importance as a clone of nontypeable *Streptococcus pneumoniae* had been implicated in sporadic cases of conjunctivitis. Use of besifloxacin and related antibiotics in combination had proved effective, but the technology and administration of this potent remedy is not available to the rural dwellers were the disease is still prevalent. Therefore, suggestions are made to provide medical facilities to all and sundry and also to enforce rational drug use and laboratory investigation before any prescription and administration.

## INTRODUCTION

Bacterial conjunctivitis which occurs in patients of all ages is characterized by itching of the eyelid, grittiness on waking, infection of the lash follicle and redness of the eye (Tarabishy and Jeng, 2008). The infection is highly contagious, being easily transmitted by contaminated fingers, towels and handkerchief and contact with infected genital discharges (Baron, 1990; Duguid *et al*., 1980; Willey *et al*., 2008). The spectrum of organisms causing conjunctival disease varies around the world. Marrow and Abbott (1998) and Olatunji (2004) reported that *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus* sp are common causes of non-gonococcal bacterial conjunctivitis. The proportion of viral and allergic conjunctivitis has largely increased in recent years, but the share of bacterial conjunctivitis in the ophthalmological incidence still remain high, about 33% (Fox *et al*., 1995). In a neonatal intensive care unit, the most common organisms isolated in patients with conjunctivitis were coagulase negative *Staphylococci*, *S. aureus* , *Neisseria gonorrhoea* and *Klebsiella* sp (Olatunji, 2004; Willey *et al*., 2008). Brooks (2008) and Iwalokun *et al*. (2011) reported mixed cultures of *Pseudomonas*, *Corynebacterium* sp, *Klebsiella*, *Enterobacter aerogenes*, *Escherichia coli*, *S. aureus*, *Moraxella* sp, coagulase negative *Staphylococci* and *Chlamydia trachomatis*.

The bacteriological and plasmid analysis of some causal agents of conjunctivitis has been investigated by (Iwalokun *et al*., 2011). Willey *et al*. (2008) and Baron (1990) had reported on the increasing multiple antibiotic resistance of *Staphylococcus aureus* and *Neisseria gonorrhoea*. A healthy conjunctiva is necessary for the maintenance of a healthy and functional cornea. Infection of conjunctivitis and related ophthalmological diseases may spread to the cornea and results in keratoconjunctivitis, inclusion conjunctivitis and ulcer because of close proximity to the conjunctiva (Willey *et al*., 2008).

This paper reports on bacterial conjunctivitis and their response to some antibiotics among patients in the eye care unit of the Federal Medical Centre, Owerri, Imo State, Nigeria.

## MATERIALS AND METHODS

### Collection of specimen

The tip of sterile swab sticks moistened with sterile physiological saline (0.9% NaCl) was used to collect profuse discharges from 60 (4 neonates, 21 adult female and 35 adult males) confirmed conjunctival patients that visited the Federal Medical Centre, Owerri, Imo State. Specimens were collected by gently rubbing the swabs along the lower fornix of the conjunctiva.

**Inoculation of specimen**

Swabs loaded with the specimen was gently streaked onto freshly prepared surface dried media (blood agar, chocolate agar, nutrient and MacConkey agar) and incubated at $37^0$C for 48h (Cheesbrough, 2000; Ogbulie and Ojiakor, 2003).

**Identification if bacterial isolates**

Pure cultures were screened on the basis of colonial, microscopic and biochemical characteristics of the isolates (Beishir, 1987; Cheesbrough, 2000) and the identities confirmed with reference to standard manuals (Buchannan and Gibbon, 2000; Cowan, 1976).

**Antibiotics screening**

Disc agar diffusion method described by Kirby-Bauer was adopted to determine antibiotic sensitivity test (Duguid *et al*., 1980). Twenty four old broth cultures of the identified bacteria were standardized (McFarland 0.5) and moistened with a sterile swab stick, and then gently streaked on the surface of freshly prepared Mueller Hinton agar, and allowed to stand for 30 minutes on the bench. Commercial antibiotic disc (Oxoid) of different concentrations was firmly placed equidistant from each other on the outer portion of the culture plate. Plates with the disc were allowed to stand for 3h and later incubated for 24-48h at $37^0$C. Zones of inhibition measured in diameter were recorded after incubation.

**RESULTS**

Colonial morphology and microscopic and biochemical characteristics of the bacterial isolates are shown in Tables 1 and 2 respectively. The predominant bacterial isolates include; *Staphylococcus aureus* (28.12%); *Streptococcus pneumoniae* (23.08%); *Haemophilus aegyptuis* (19.23%). *Neisseria gonorrhoea*, *Corynebacterium* and *Escherichia coli* were also isolated, representing 8.97%, 12.82% and 7.69% respectively (Table 3).

*Staphylococcus aureus* was sensitive to gentamycin, streptomycin and cotrimoxazole but showed high resistance to nitrofurantoin, colistin sulphate, ampicillin, nalidixic acid and tetracycline. *Streptococcus* sp was sensitive to nalidixic acid, streptomycin and nitrofurantoin but resistance to colistin sulphate, tetracycline and cotrimoxazole. *Neisseria gonorrhoea* and *Corynebacterium* species were sensitive to ampicillin and tetracycline. All the bacteria isolated from the infected patients were resistant to chloramphenicol (Table 4).

The enterobacteriaceae species generally showed resistance to ceporex and zinnate. Gentamycin was sensitive against 52.6% of all the isolates, whereas streptomycin was effective against over 57.7% of the species of the six genera isolated. Zinnate was sensitive against 56.4% of the isolates while nitrofurantoin was effective against 38.5% of the isolates. Cotrimoxazole was effective against 53.9% of the isolates (Table 4).

**Table 1. Colonial and microscopic characteristics of bacterial isolates from conjunctival specimens**

| Colonial characteristics | Grams reaction | Capsule | Spores | Motility | Probable identity |
|---|---|---|---|---|---|
| Smooth moist and shiny golden yellow colonies | Gram positive cocci in predominantly in clusters, few in tetrads and pairs | - | - | - | *Staphylococcus* sp |
| Small circular cream moist and shiny colonies | Gram positive diplococcic in chains | + | - | - | *Streptococcus* sp |
| Smooth moist and shiny pinpoint colonies | Small gram negative rods in singles and few in pairs | - | - | - | *Haemophilus* sp |
| Cream dull and dry serrated colonies | Gram negative oval and bean shaped colonies in pairs | - | - | - | *Neisseria* sp |
| Dull and dry cream umbonate shaped colonies | Gram positive pleomorphic rods with the shape of Chinese letters | + | - | - | *Corynebacterium* sp |
| Small circular moist and shiny colonies | Gram negative rods predominantly in singles | - | - | + | *Escherichia coli* |

**Table 2. Biochemical and Carbohydrate characteristics of bacterial isolates**

| Cat | Oxi | Coag | In | MR | VP | Cit | Lect | Hae | Ure | Glu | Lac | Man n | Suc | Mal | Identity of isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | - | + | - | - | + | - | - | + | + | +(A) | +(A) | +(A) | +(A) | +(A) | *Staphylococcus aureus* |
| - | - | - | - | - | + | - | - | + | - | +(A) | - | - | - | +(A) | *Streptococcus pneumoniae* |
| + | - | - | - | - | + | - | + | + | - | A | - | - | - | +(A) | *Haemophilus aegyptius* |
| + | + | - | - | + | - | + | + | - | - | +(A) | - | A | A | - | *Neisseria gonorrhoea* |
| + | - | - | - | - | + | + | - | - | - | +(A) | +(A) | - | +(A) | +(A) | *Corynebacterium sp* |
| + | - | - | + | - | + | - | - | - | - | +(A) | +(A) | A | - | A | *Escherichia coli* |

Cat, catalase; Oxi, oxidase; In, indole; MR, Methyl Red; VP, Voges, Proskaeur; Cit, citrate; Lect, Lecithinase; Hae, Haemolysis; Ure, Urease; Glu, Glucose; Lac, Lactose; Mann, Mannitol; Suc, Sucrose; Mal, Maltose

**Table 3. Prevalence of bacteria of probable pathogenicity in conjunctival specimens**

| **Bacterial isolates** | **No. of isolates** | **Percentage of isolates (%)** |
|---|---|---|
| *Staphylococcus aureus* | 22 | 28.21 |
| *Streptococcus pneumonia* | 18 | 23.08 |
| *Haemophilus aegytuis* | 15 | 19.23 |
| *Neisseria gonorrhea* | 7 | 8.97 |
| *Corynebacterium* spp | 10 | 12.82 |
| *Escherichia coli* | 6 | 7.69 |
| Total | 78 | 100 |

**Table 4. Sensitivity pattern of isolates to some selected antibiotics**

| Isolates | No. of isolates (%) | GEN | NIT | COL | CAZ | CXM | CL | AMP | STR | NAL | TET | COT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | 22(28.21) | 20 | 0 | 8 | 18 | 7 | 0 | 0 | 17 | 0 | 0 | 20 |
| *Streptococcus pneumoniae* | 18(23.08) | 2 | 10 | 7 | 5 | 5 | 0 | 1 | 17 | 10 | 0 | 0 |
| *Haemophilus aegyptius* | 15(19.23) | 11 | 10 | 5 | 10 | 6 | 0 | 0 | 0 | 10 | 8 | 13 |
| *Neisseria gonorrhoea* | 7(8.97) | 4 | 3 | 2 | 4 | 2 | 0 | 5 | 2 | 4 | 6 | 4 |
| *Corynebacterium* sp | 10(12.82) | 4 | 5 | 3 | 3 | 2 | 0 | 8 | 4 | 4 | 9 | 0 |
| *Escherichia coli* | 6(7.69) | 0 | 2 | 4 | 4 | 2 | 0 | 4 | 5 | 0 | 6 | 5 |
| Overall % sensitivity | 78 | 41(52.6) | 30(38.5) | 29(37.2) | 44(56.4) | 24(30.8) | 0 | 18(23.1) | 45(57.7) | 28(35.9) | 29(37.2) | 42(53.9) |

GEN, Gentamycin (10µg); NIT, Nitrofurantoin (200µg); COL, Colistin sulphate (25µg); CAZ, Zinnate (10µg); CXM, Ceporex (25µg); AMP, Ampicillin (25µg); STR, Streptomycin (25µg); NAL, Nalidixic acid (30µg); TET, Tetracyclin (25µg); COT, Cotrimoxazole (25µg); CL, Cloramphenicol (25µg).

## DISCUSSION

The spectrums of bacteria isolated from conjunctival swabs were similar to the reports of others (Baron, 1990; Duguid *et al*., 1980; Friedlaender, 1995; Merianos *et al*., 1995; Willey *et al*., 2008). *Staphylococcus aureus*, *Haemophilus aegyptus* and *Streptococcus pneumoniae* were the most predominant. *Neisseria gonorrhea, Escherichia coli* and *Corynebacterium* species were also isolated.

A proportion of the bacterial isolates were resistant to some commonly used antibiotics. *Staphylococcus aureus* for instance recorded 100% resistance to nitrofurantoin, ceporex, ampicillin, nilidixic acid, tetracycline and cotrimoxazole. This corroborate with the findings of Baron (1990) and Bravenly and Milatovic (1987).

*Streptococcus pneumoniae*, one of the most frequent isolates was 100% resistance to chloramphenicol, tetracycline and cotrimoxazole. High resistance of *Streptococcus pnuemoniae* to ampicillin (94.44%) and gentamycin (89%) had been reported by Marrow and Abbott (1998).

Some of the isolates, notably, *Escherichia coli* and *Staphylococcus aureus* may results from contamination or as commensals from the face and skin of many carriers with depressed immunity (Jones *et al*., 1995).

*Neiserria gonorrhoea* isolated from the eyes of neonates is sexually transmitted and highly contagious pathogens (Willey *et al*., 2008). Gonoccocal ophthalmia also known as ophthalmia neonatorum is a disease condition in newborns infants transmitted through the birth canal of an infected mother (Duguid *et al*., 1980; Willey *et al*., 2008). *Haemophilus aegyptus* also known as "Koch-Weeks bacilli" is a commensal of the upper respiratory tract and is frequently isolated during epidemic involving camps and school environment (Baron, 1990). *Corynebacterium* species, though a part of the normal flora of the eye may become pathogenic when the immune system is compromised. Production of toxins contributes to the virulence of *Corynebacterium* species (Baron, 1990).

Greenhood *et al*. (2003) reported that penetrating injuries of the eyes and ophthalmic surgery may introduce a wide range of bacteria and fungi into the chambers of the eye which may give rise to hypopyon (pus in the eye). Choroidoretinitis, an infection of the back of the eye are seen in many diverse infectious diseases associated with immunocompromised individuals (Greenhood *et al*., 2003; Willey *et al*., 2008).

The high resistance of the bacteria to some narrow and broad spectrum antibiotics is worrisome giving the fact that the eye is usually exposed and susceptible to infections from dust and contaminated skin surfaces. Uncontrolled prescription of antibiotics by health workers and their indiscriminate use may account for the drug resistance resulting from mutant strains.

With the pattern of resistance to some of the antibiotics under investigations, the habit of drug abuse for the blind treatment of the infections, especially for the diseases of the eyes should be discouraged while the use of new and more effective antibiotics should be recommended. Besifloxacin a broad spectrum fluoroquinone has been reported as a novel anti-infective agent in the treatment of bacterial conjunctivitis (Comstock *et al*., 2009; Comstock *et al*., 2010; Haas *et al*., 2009; McDonald *et al*., 2009; Paterno *et al*., 2009).

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