ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF \textit{Pseudomonas aeruginosa} ISOLATED FROM CONTACT LENSES OF SOME SELECTED INDIVIDUALS IN CALEB UNIVERSITY, IMOTA, LAGOS STATE

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ABSTRACT

Contact lenses are ocular prosthetic devices used by over 150 million people worldwide, and they can be worn to correct vision, for cosmetic, or therapeutic reasons. This epidemiological study was performed to determine the prevalence of \textit{Pseudomonas aeruginosa} on contact lenses of selected students in Caleb University, Imota, Lagos State. A swab of 100 contact lenses from selected volunteers comprising 43 males and 57 females were collected for microbiological examination. Sterile swab sticks moistened with sterile distilled water were used to aseptically swab the lenses and transferred into nutrient broth followed by incubation for 5 hours. Thereafter, the resultant broth culture was subcultured on to cetrimide nutrient agar and incubated at 37\textdegree C for 24 hours for the isolation of \textit{Pseudomonas aeruginosa}. A total of 30 (30\%) of the contact lenses sampled were found to have \textit{Pseudomonas aeruginosa}, of which 13(43.3\%) of the isolates were pigmented and 17 (56.7\%) were non-pigmented. Eighty percent (80\%) of the isolates were susceptible to ciprofloxacin, 75\% to gentamicin while 50\% were susceptible to ofloxacin. The isolates were 90\% resistant to nitrofurantoin, 75\% resistant to augmentin and 100\% to ampicillin. Ciprofloxacin and gentamicin were found to be effective against \textit{Pseudomonas aeruginosa} in this study and could be of therapeutic relevance for the management of contact lenses mediated infections.

KEYWORDS: Antimicrobial susceptibility; Prevalence; Contact lenses; \textit{Pseudomonas aeruginosa}.

INTRODUCTION

Eyes are organs of the visual system. They detect light and convert it into electrochemical impulses in neurons. In higher organisms, the eye is a complex optical system which collects light from the surrounding environment, regulates its intensity through a diaphragm, focuses it through an adjustable assembly of lenses to form an image, converts this image into a set of electrical signals and transmits these signals to the brain through complex neural pathways that connect the eye via the optic nerve to the visual cortex and other areas of the brain\cite{1}. Because of this visual perception we are able to know what is happening around us in our external environment and accordingly we are able to act towards a particular situation. But sometimes these eyes may become defective due to various reasons. Various common defects of the eyes; the problems they pose to humans in their vision, the cause of these defects and the possible ways to overcome these defects include the wearing of contact lenses \cite{2}.

Contact lenses constitute a popular means of vision correction and are worn by approximately 85 million individuals worldwide. A new lens daily in respect of
disposable wears, extended wear for beyond one day up to one week before replacement involving treating with disinfectants at night and/or continuous for one month before replacement depending on the purpose and individual convenience [3]. Contact lenses are worn on the eyes by people to enhance sight, correct a defect of the eye, protect eye from sunlight or for fashion. The alarming prevalence of recommended glass wearing that cut across both the young and the aged can be attributed to many factors. These factors include the decline immunity in adults, eye infection which may probably be hereditary, environmental influence, abuse of eye caring norms and non-adherence to personal hygiene. Studies have shown that bacteria do adhere to contact lenses and that the extent of colonization depend on the nature of the material used. It has been shown that the oxygen content of the tear film underlying the lens may be markedly reduced depended on the nature of the material used in the manufacture of contact lenses [4]. The pivotal interrelationship between the blink reflex (usually 12 blinks per minute), rubbing of the eye, moving over the cornea to remove desquamated cells with any associated microbes coupled with the environment provide an ease of contamination of contact lenses with potential pathogens even during the ocular antimicrobial defense mechanisms. Majority of contact lenses wearers used either rigid gas-permeable or soft hydrogel lenses. It is relevant to know that environmental organism a nutritionally non exacting Pseudomonas aeruginosa is an efficient colonizer of many types of contact lenses material, and its viability is unaffected by many contact lenses disinfecting solutions [5]. It can therefore, form a biofilm on the contact lenses. Many contact lenses wearers have an increased risk of developing microbial keratitis, and in 70% of such cases, the causative agent is Pseudomonas aeruginosa [6]. Caleb University is a private university situated at Imota, Ikorodu axis of Lagos State with population of 3000 peoples of varied age and gender. The aim of this studies was to collect swab samples from contact lenses of volunteers using sterile swab sticks to cultivate the samples on cetrimide nutrient agar (a selective medium designed for the isolation of Pseudomonas aeruginosa), to characterize the isolates biochemically and carry out antibiogram on the isolates for the purpose of discovering antibiotics of relevance for the management of pseudomonads-mediated eye infections.

**MATERIALS AND METHOD**

**Target population**

The population size of Caleb University, Imota, Lagos comprising of students from all colleges of studies, lecturers and non-teaching staff as well as canteen workers of about 3000 individuals were randomly sampled.

**Collection of samples**

A total of 100 swabs of contact lenses from volunteers’ wearers varied in age and gender were collected within the University premises.

**Bacteriological isolation**

Sterile swab sticks moistened with sterile distilled water were used to aseptically swab the contact lenses and the swab were transferred into sterile nutrient broth prior to subculture on to cetrimide nutrient agar (CNA). The culture was then incubated at 37°C for 24 hours for the isolation of Pseudomonas aeruginosa.

**Identification of isolates**

The bacterial isolates actively growing on the selective CNA media were subjected to conventional biochemical characterization to confirm the isolates as Pseudomonas aeruginosa. The biochemical tests carried out were; Gram staining, oxidase test, citrate utilization test, glucose, sucrose and lactose fermentation test.

**Antibiogram**

Antibiotic sensitivity test was determined by modified disk diffusion technique on the biochemically characterized isolates. A volume of 0.1mL of the overnight broth culture of every isolate was pipetted into 9.9mLs of sterile distilled water in the test tubes to make 10⁻² dilution of the organism. From this dilution, 0.1mL of the diluted culture was pipetted into the sterile melted and cooled (45 °C) Mueller Hinton Agar medium (Oxoid) and aseptically poured into the sterile culture plate and were allowed to set, the antibiotic multi-discs were aseptically placed in each plate, after 1hr of pre-diffusion, they were then incubated at 37°C for 24 hours. The zones of growth inhibition were recorded, analyzed and interpreted according to CLSI standard.
RESULTS

The results are presented in Table 1 and Figure 1. The age distribution range was grouped within an interval of 12 ages and the highest number of the samples (33) were collected within the age 13 - 24 years which yielded 9 isolates of *Pseudomonas aeruginosa*. Age range 25 - 36 yielded 7 isolates while 8 isolates were obtained from 25 samples collected from the age group of 49-60 years. No isolate was present in the age group 0-12 years and above 60 years respectively as shown in Table 1. The biochemical characterization of the 30 Gram stained isolates of *Pseudomonas aeruginosa* obtained in this study were citrate and oxidase positive and none of the isolates fermented glucose, maltose and sucrose sugars.

DISCUSSION

*Pseudomonas* is the most common bacteria in contact lenses because it adheres to contact lens surfaces more easily than many other pathogens. More specifically, *Pseudomonas aeruginosa* often exhibit pili and flagella that can facilitate the adhesion processes. Complicating this association, there is a non-piliated *Pseudomonas aeruginosa* strain that easily can adhere to the contact lens surface as well [7]. Within 24 hours of exposure, *Pseudomonas* typically form a biofilm with the contact lens, which causes permanent, irreversible surface adhesion. Some researchers believe that this occurs due to surface hydrophobicity [7, 8].

In this study, contact lenses from one hundred volunteers were assessed, from which 30 isolates of *Pseudomonas aeruginosa* were identified. Thirteen (13) isolates elicited greenish pigmentation while the remaining 17 isolates showed no pigmentation which could be due to their genetic variation. More samples were collected from female participants (57%) than male (43%). The different numbers of isolates of *Pseudomonas aeruginosa* isolated from the contact lens samples may be a reflection of either the single use or continuous use of the respective contact lenses, the health status of the wearers, the environmental influence, individual approach to hygiene awareness and their understanding about the intricacies associated with eye related issues [9].

The 30 isolates of *Pseudomonas aeruginosa* biochemically characterized in this study were oxidase and citrate positive but none of the isolates fermented glucose, maltose and sucrose. However, the results of biochemical tests obtained are typical of *Pseudomonas aeruginosa* which could be due to the genetic constitution as well as their conventional inherent metabolic mechanisms. This agrees with the studies of Harold JB, 2002 on biochemical profiling of *Pseudomonas aeruginosa*.

The alarming resistance pattern to ampicillin, augmentin and cefuroxime in this study is a known phenomenon though augmentin can be said to be a broad-spectrum antibiotic, so also cefuroxime. The increasing resistance of *Pseudomonas aeruginosa* could be extrinsic or intrinsic depending on the prevailing phenomenon. Sixty-three (63%) were resistant to ceftazidime, while (65%) of the isolates were resistant to cefuroxime. Twenty-five (25%) of the isolates were found to be resistant to gentamicin while (20%) were resistant to ciprofloxacin, 50% resistance to ofloxacin while 75% were resistant to augmentin. The highest resistance to nitrofurantoin (90%) and ampicillin (100%) were recorded in this study. Resistance in this study could be due to excessive beta lactamase production or efflux mechanism, hardy nature of *Pseudomonas aeruginosa* that is closely related to its impermeability to antimicrobial agents offer by the outer membrane as well as its periplasmic spaces which is known to contain antimicrobial inactivating enzyme. Also, inappropriate use of antibiotics, poor prescription and inclusion of antibiotics in consumable agricultural products could be responsible for the resistance obtained since the isolates used were from different individuals with varied hygiene status, though in the same environment.

Isolation of *Pseudomonas aeruginosa* from contact lenses in this study was an indication that contact lenses can act as a vector for introducing microbial pathogens to the eye. A study carried out in North America, reported that the incidence of microbial keratitis secondary to *Pseudomonas* is 2.76 cases per 10,000 individuals a year. However, when considering only contact lens wearers, this number increases dramatically to 13.04 cases per 10,000 individuals a year. In other words, contact lens patients are more than nine times as likely to develop a *Pseudomonads*-mediated infection as those who don’t wear contacts, and most of the strains are resistant to antimicrobial agents [10].

In conclusion, the phenomenon of resistance recorded in this study could be attributed to relatively nutritionally non-exacting nature and genetic makeup of *Pseudomonas aeruginosa*, which permits it to survive fairly easily in a variety of outdoor and indoor environments and its extrinsic and intrinsic armaments which are pivotal to antimicrobial resistance. Therefore, proper hygiene should be observed in wearing contact lenses and cleaning of contact lenses biofilms with disinfectant of standard concentration to prevent colonization and
Table 1: Age and sample distribution

<table>
<thead>
<tr>
<th>Age group</th>
<th>No of sample examined</th>
<th>Positive (isolates)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13-24</td>
<td>33</td>
<td>9</td>
<td>27.3</td>
</tr>
<tr>
<td>25-36</td>
<td>25</td>
<td>7</td>
<td>28.0</td>
</tr>
<tr>
<td>37-48</td>
<td>11</td>
<td>6</td>
<td>54.5</td>
</tr>
<tr>
<td>49-60</td>
<td>26</td>
<td>8</td>
<td>30.8</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>30</strong></td>
<td><strong>30</strong></td>
</tr>
</tbody>
</table>

% of isolates obtained from the samples = \( \frac{\text{Number of isolates obtained}}{\text{Sample collected}} \times 100 \)

Figure 1: Bar chart showing Percentage susceptibility of the isolates to antibiotics.

Key: CAZ- ceftazidime, CRX- cefuroxime, GEN- gentamicin, CPR- ciprofloxacin, OFL- ofloxacin, AUG- augmentin, NIT- nitrofurantoin, AMP- ampicillin
development of antimicrobial resistance. Medical regular check-up could be an added preventive measure [11]. However, essential knowledge regarding contact lens and its accessories and hygienic practice when handling them and their casing is imperative to prevent or reduce undesirable pseudomonads colonization.

REFERENCES

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