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Isolation and identification of bacterial isolates associated with spectacle lens surface

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Abstract

People nowadays suffer from refractive disorder that declines the vision. The most common therapy to overcome this challenge is the use corrective spectacle lenses. The continuous use of spectacles and the difficulties on disinfecting the entire surface can cause bacterial contamination, those bacteria can cause various eye diseases. This study aims to isolate and identify the bacterial isolates associated with spectacle lens surface. The samples were then inoculated on nutrient and macConkey agar and incubated at 37°C for 24 hours. Then, the isolates were identified macroscopically and microscopically. 72 out of 100 samples indicated positive results with four (4) different identified bacterial species of *Staphylococcus aureus* (38.89%), *Pseudomonas aeruginosa* (27.78%), *Klebsiella pneumonia* (22.22%), and *Escherichia coli* (11.11%). The result of this study revealed the presence of bacterial isolates on spectacle lens surface. All the identified bacterial species are pathogenic to the eye. This indicated that, spectacle lenses could serve as a reservoir of pathogenic bacterial specie.

Keyword: Bacterial isolates; Spectacle lens; Lens surface; MacConkey agar; Nutrient Agar

1. Introduction

According to [1], spectacle lenses are the most commonly used solutions for refractive errors of the eye, such as myopia, hyperopia, presbyopia and astigmatisms, to correct a refractive error, the spectacle lens needs to have the correct lens power and an appropriate distribution of power along the surface of the lens. Eye lenses are glasses or plastic optical items that fit inside eyewear frames to enhance and/or correct the wearer's vision, it is also regarded as inanimate object.

Microorganisms are group of living things that are too small to be seen with the naked eye, but through the help of an optical instrument called microscope. They are highly ubiquitous and nearly found everywhere. Microorganisms can either be beneficial or harmful to human-being, they also have the ability of causing a number of diseases or ailments [2].

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Ocular infections are common [3,4] and have been associated with microbial contamination of contact lenses and eyeglasses [5,6]. Many frequently used devices, such as eyeglasses and contact lenses, are rarely sterilized or kept clean, which increases the likelihood of bacterial contamination and colonization [7,8]. Significant number of people wear eyeglasses for functional or purely cosmetic reasons [9]. The human eyes have a special anatomy that make them vulnerable to infectious agents, including bacteria, as well as direct environmental exposure. The eyelids, conjunctiva, and cornea are the main parts of the eyes that are most frequently infected. Several ocular diseases, including conjunctivitis, keratitis, scleritis, choroiditis, iridocyclitis, retinal vasculitis, retinitis,and posterior uveitis, have been linked to contamination of handles and lenses of eyeglasses. This is quite worrisome, because these bacteria are transferred to the eyewear through contact with infected hands and other methods [6].

According to [10], microorganisms are transmitted from animated sources to inanimate environ- mental sources, which may become secondary reservoirs if they meet the needs of transmitted pathogens to survive and to multiply. In healthcare settings, however, contaminated surfaces, which may not always be optimal for microbial survival and multiplication, still may play a role in the chain of infection, since surfaces close to the patients' environment may be touched at high frequencies, allowing transmission from animated sources to others via contaminated inanimate surfaces.

[10] also said the risk for transmission of Hospital Acquired Infection (HAI) depends of the persistence of nosocomial pathogens on surfaces. The longer a microorganism may persist on a surface, the longer the contaminated surface may be a source of transmission and thus endanger a susceptible patient or healthcare worker of becoming the target of infection.

Many Gram-negative species, such as *Acinetobacter spp., Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa, Serratia marcescens, or Shigella spp.* can survive on inanimate surfaces even for months [10]. These species are found among the most frequent isolates from patients with HAI [11]. However, a few others Gram-negative bacteria, such as *Bordetella pertussis, Haemophilus influenzae, Proteus vulgaris, or Vibrio cholera* persist only for days [11].

Mycobacteria, including Mycobacterium tuberculosis, and spore-forming bacteria, such as *Clostridium difficile*, can survive for many months on surfaces [10].

2. Material and methods

2.1. Study Area

The study was conducted at Aminu Dabo College of Health Sciences and Technology, and Aminu Dabo College of Nursing Sciences Kano-Nigeria.

2.2. Ethical Clearance

An approval for the study was obtained from the research ethics committee of Aminu Dabo College of Health Sciences and Technology and Aminu Dabo College of Nursing Sciences Kano-Nigeria. Kano.

2.3. Study Design

The study is an experimental study design

2.4. Study Population

One hundred (100) spectacle lenses from both students of Aminu Dabo College of Health Sciences and Technology and Aminu Dabo College of Nursing Sciences Kano-Nigeria were engaged in this study.

2.5. Sample Collection and Processing

A questionnaire was administered to each student and the sample was taken instantly by using swab stick moistened in sterile distilled water and swabbed on the lens surface, the samples were well labelled and transported immediately to the Microbiology Laboratory of Aminu Dabo College of Health for processing.

2.6. Isolation of Bacterial Isolates

The sterile swabs containing the samples was spread on the surface of Nutrient agar and incubated for 24 hours at 37°C, the isolated colonies were sub-cultured for another 24 hours at 37°C using MacConkey agar according to standard procedure as reported by [12].

2.7. Identification and Classifications of Bacterial Isolates

The following biochemical tests were carried out for the classifications and identification of the organisms: Gram's stain for classifications of bacterial isolates, catalase test, coagulase test and indole tests for identifications of bacterial isolates.

2.7.1. Gram's Staining

This test was done according to [13]. The Gram stain is by far the most widely used procedure for staining bacteria and separating it into two major groups: Gram (+) positive and Gram (-) negative.

A thin film of specimen was spread over a clean grease free slide and allowed to air dry. Fixed it by passing it over a Bunsen flame thrice. Flooded the film with crystal violet and leave for 60 seconds. The slide washed off and flooded the stain with lugol's iodine and (mordant) and leave for 60 seconds. Washed off iodine and decolorized the slide with acetone (decolorizer) for a second, washed the slide and train with safranin (counter stain) for 60 seconds and washed off. The back of the slide was air dried. Examined with the oil immersion, x 100 lens. A purple color signified Gram (+) positive while the color of the safranin which is red signifies Gram (-) Negative.

2.7.2. Catalase Test

This test was done according to [14]. The test was performed by dropping a loop of the isolate mixed with the hydrogen peroxide on the slide. The production of gas bubbles (02) from the mixture occurred almost immediately indicating a positive reaction.

$$2H202 \longrightarrow 2H20 + 02$$

2.7.3. Coagulate Test

This test was done according to [14] to differentiate staphylococcus aureus and other staphylococcus species. 2 - 3 drops of normal saline were added on a grease free slide to the normal saline and mixed the suspected organism and 1 – 2 drops of plasma was added and Rock, the presence of agglutination indicated a positive result.

2.7.4. Indole Test

This test was done according to [15] to differentiate members of enterobacteriacea, *Escherichia coli* is indole positive and only some shigella strain are indole positive.- The test organism was inoculated in a test tube containing 3ml of sterile tryptone water and incubated at 37oc for 24hrs. The test for indole was done by adding 0.5ml of kovac's reagent and shaken gently. The presence of a red color in the surface of the layer within 10minutes indicated positive result.

2.8. Result Presentation

The result was presented using tables.

2.9. Result Analysis

The result was analyzed by using Chi-square.

3. Results

Out of the 100 samples collected from spectacle lens surface, 72 number of samples indicated positive result with four (4) number of different bacterial species of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli* out of which *Staphylococcus aureus* have the highest incidence with 28 number of isolates out of 72 isolates representing 38.89%. *Pseudomonas aeruginosa* have the second incidence with 20 number of isolates representing 27.78%. *Klebsiella pneumonia* have the third incidence with 16 number of isolates representing 22.22% while *Escherichia coli* have the least incidence with 8 number of isolates representing 11.11% as shown in table 1.

Table 1 Bacterial isolates identified from spectacle lens surface

S/N	Organisms	No. of isolates	% of Occurrence
1	*Staphylococcus aureus*	28	38.89
2	*Pseudomonas aeruginosa*	20	27.78
3	*Klebsiella pneumonia*	16	22.22
4	*Escherichia coli*	8	11.11
	TOTAL	72	100

Key: No: Number, %: percentage

Out of the four (4) different species isolated and classified from the spectacle lens surface using gram staining techniques, only *Staphylococcus aureus* (coagulase positive) was identified as Gram-positive bacteria, while the remaining bacterial species of *Pseudomonas aeruginosa* (catalase positive), *Klebsiella pneumonia* (catalase positive) *and Escherichia coli* (indole positive) were all identified as Gram-negative bacteria as shown in table 2.

Table 2 Identification and Classification of Bacterial Species Isolated from Spectacle Lens Surface Using Gram Staining Techniques

S/N	Bacterial specie	Gram positive	Gram negative
1	*Staphylococcus aureus* (Coagulase positive)	Yes	No
2	*Pseudomonas aeruginosa* (Catalase positive)	No	Yes
3	*Klebsiella pneumonia* (Catalase positive)	No	Yes
4	*Escherichia coli* (Indole positive)	No	Yes

According to the duration of use, students who uses spectacles for more-than two years were highest in number with 40 number of students, out of which four (4) different species of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli* were isolated from their samples, while those who uses spectacle from 0-1 year were second in number with 34 number of students out of which three (3) bacterial species of *Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli* were identified, and those who uses spectacle from 1-2 years were least in number with 26 students out of which two bacterial species of *Staphylococcus aureus,*and *Pseudomonas aeruginosa* were identified as shown in table 3.

Table 3 Bacterial Species Isolated from Spectacle Lens Surface in Relation to Use Duration

Duration of use	No. of students	No. of species identified	Name of identified species
0-1 Year	34	3	*S. aureus, K. pneumoniae, E. coli*
1-2 Years	26	2	*S. aureus, P. aeruginosa*
2-above years	40	4	*S. aureus, P. aeruginosa, K. pneumoniae E. coli*
TOTAL	100		

Key: No: Number; Chi-square: 0.08736; Degrees of Freedom: 2; Table size: 3 rows, 2 columns; The P value is 0.9573.

The row and column variables are not significantly associated.

According to gender of the 100 students participated, 65 were females representing 65% while 35 were males representing 35%, females have the highest incidence with 48 number of their samples showed positive results

representing 66.66% and 17 number representing 60.71% were negative, while males have the lowest incidence with 24 number of samples being positive representing 33.33% and 11 number representing 39.28% were negative as shown in table 4.

Table 4 Bacterial Agents Isolated from Spectacle Lens Surface in Relation to Gender

Gender	No. of students	No. of positive result (%)	No. of negative result (%)
Males	35	24 (33.33)	11 (39.28)
Females	65	48 (66.66)	17 (60.71)
TOTAL	100	72 (100)	28 (100)

Key: No: Number, %: percentage; Chi-square: 0.3140;; Degrees of Freedom: 1; Table size: 2 rows, 2 columns; The P value is 0.5753.; The row and column variables are not significantly associated.

4. Discussion

Out of the one hundred (100) samples analyzed in this study, seventy-two (72) number of samples were found positive representing (72%) unlike the works of [16] and [17] who reported bacterial contaminants in 100% of the eye glasses examined, but agrees with the works of [5] and [18] who reported bacterial contaminants in less-than 100% of the eye glasses examined i.e 95% and 93.4% respectively.

The result of isolation and identification of bacterial isolates associated with spectacle lens surface revealed that, out of the four (4) number of the identified species which includes: *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli. Staphylococcus aureus* was the most predominant specie with total number of 28 isolates representing 38.89%, this agrees with the work of [17] who works on bacterial pathogens associated with eye glasses and risk of infection and found out that *Staphylococcus aureus* was the highest isolated organism with 54%. This result also agrees with the work of [16] who work on bacterial contaminants on glasses used by students and concludes that *Staphylococcus aureus* is the bacteria that grows well on human skin, the part of the human body that often becomes the site of *Staphylococcus aureus* is the front nasal cavity or anterior nares. *Staphylococcus aureus* is often found in the area around the eye [19]. According to [16] *Staphylococcus aureus* can cause various kinds of infections in human, including infection of the eye, this study suspected that *Staphylococcus aureus,* contaminates spectacle lens through the handkerchief used to clean the nose and at the same time which some students use in lens cleaning.

The emergence of *Pseudomonas aeruginosa* in this study, as the second most predominant isolate with 27.78% is in agreement with the work of [16] in which *Pseudomonas aeruginosa* became the second highest number of bacteria isolated in their study with 46.66% and concludes that *Pseudomonas aeruginosa* is the bacteria that grows well in a variety of environment. According to [20] *Pseudomonas aeruginosa* can be transmitted through direct contact with water, and these bacteria can even be found in disinfectant fluids in hospitals. In the clinical environment, *Pseudomonas aeruginosa* known to cause opportunistic infections [21]. According to [22] *Pseudomonas aeruginosa* as a contaminant on glasses can cause various diseases of the eye, such as corneal ulcers. *Pseudomonas aeruginosa* is the most common bacteria found to be the cause of Keratitis in contact lens wearers [23].

Moreover, the emergence of *Escherichia coli* as the least isolated organism in this study with 11.11% is not in agreement with the work of [18] who reported *Escherichia coli* as the highest isolated bacterial specie in their study with 51.9%. Also, the emergence of *Klebsiella pneumoniae* as the second to the least isolated bacterial specie in this study with 22.22% is in contrary to the work of [18] who reported *Klebsiella pneumoniae* as the least isolated specie in their study with 1.89%.

Furthermore, the result of bacterial classifications revealed that, out of four (4) identified species, only *Staphylococcus aureus* is gram positive bacteria while the remaining three (3) species of *Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli* were gram negative, this result agrees with the work of [17 and 16] who reported the isolation of both gram positive and gram negative bacterial species from their studies.

In relation to duration of use, the result of present study revealed that, students who uses spectacle for 2 and above years have more number of positive results in which all the four (4) species of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli* were found in their samples. This shows that, the survival of microorganisms especially bacterial isolates on spectacle lens surface is highly related to the duration and level of usage, this result agrees with [24] who reported bacterial isolates can survive on surfaces for long period of time.

According to the gender of students, female spectacle lenses have more number of bacterial isolates in this study with 48 out of 72 positive isolates representing 66.66% compared to that of males with 24 out of 72 positive isolates representing 33.33%, this result is not in agreement with the work of [17] who reported male eye glasses have more bacterial contaminants with 54.2% compared to that of females with 47.4%.

5. Conclusion

The results of this study indicated that, there was bacterial isolates surviving on spectacle lens surface used by students of Aminu Dabo College of Health Sciences and Technology and Aminu Dabo College of Nursing Sciences respectively. In this study, there were four (4) identified species of bacteria, the *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli.* This shows that spectacle lens surface can be a reservoir of pathogenic bacteria that can potentially cause repeated infections in the eye.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors'.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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