

A Study on the Isolation and Analyzation of *Staphylococcus aureus* Obtained from Nasal Samples of Female Students (Undergraduates and Graduates) at The Obafemi Awolowo University Campus

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Received January 02, 2019; Revised February 06, 2019; Accepted February 10, 2019

Abstract One of the predominant bacteria found in the nose is *Staphylococcus aureus*, and they are found in approximately the same number as on the skin and face. The study was carried out to isolate and analyze *Staphylococcus aureus* obtained from the nasal cavity of female students (at undergraduate and postgraduate levels) of the Obafemi Awolowo University, Ile-Ife campus, Osun state, Nigeria. Out of 40 samples obtained from the nasal cavity of the female students, a total of 9 isolates were confirmed as *Staphylococcus aureus*. Based on the biochemical tests, they were all Gram-positive cocci in clusters, catalase positive, coagulase positive and were also DNase positive. Based on these criteria, we concluded that individual should ensure that their nose is kept clean and should practice good hygiene especially in a hospital environment to prevent nosocomial infections due to *Staphylococcus aureus*.

Keywords: *Staphylococcus aureus*, gram-positive cocci, DNase catalase, coagulase, catalase, nasal cavity.

Cite This Article: Taiwo Mary Makinde, Kwashie Ajibade Ako-Nai, and A. O. Shittu, "A Study on the Isolation and Analyzation of *Staphylococcus aureus* Obtained from Nasal Samples of Female Students (Undergraduates and Graduates) at The Obafemi Awolowo University Campus." *American Journal of Microbiological Research*, vol. 7, no. 1 (2019): 24-27. doi: 10.12691/ajmr-7-1-4.

1. Introduction

The visible part of the human nose is the protruding part of the face that bears the nostrils. The shape of the nose is determined by the ethmoid bone and the nasal septum, which consists mostly of cartilage that separates the nostrils. On the average, the nose of a female is smaller than that of a male (http://en.wikipedia.org/wiki/Human_nose). The nose has an area of specialized cells which are responsible for smelling (part of the olfactory system). Another function of the nose is the moderation of the inhaled air, warming it and making it more humid. The hairs inside the nose prevent large particles from entering the lungs. In addition, sneezing is usually caused by foreign particles irritating the nasal mucosa but can more rarely be caused by sudden exposure to bright light (called the photic sneeze reflex) or touching the external auditory canal. Sneezing is a major means of transmitting infections because it creates aerosols in which the droplets can harbor microorganisms [1]. The nasal root is the top of the nose, that forms an indentation at the suture where the nasal bone meets the frontal bone. The anterior nasal spine is a thin projection of the bone at the midline on the lower

nasal margin, which holds the cartilaginous center of the nose (http://en.wikipedia.org/wiki/Human_nose).

The mammalian respiratory system has a formidable defense mechanism. The average person for example, inhales at least eight microorganisms a minute or about 10,000 each day. Once inhaled, a microorganism must first survive and penetrate the air filtration system of the upper and lower respiratory tracts. Because the airflow in this tract is turbulent, microorganisms are easily deposited on the moist, sticky mucosal surface. The cilia in the nasal cavity beat towards the pharynx so that the mucus with its trapped microbes are moved toward the mouth and then expelled [2]. The normal microbiota of the nose is found just inside the nostrils. *S. aureus* and *S. epidermidis* are the predominant bacteria present and are found in approximately the same numbers as on the skin and face [2]. About 20% of healthy individuals almost never carry *S. aureus*, however, about 60% carry *S. aureus* from time to time [1].

1.1. Staphylococci

The staphylococcus is a common bacterium found on the skin and nose of the healthy individuals. The name staphylococcus is derived from a Greek word *staphyle*,

which means a bunch of grapes and kokkos which means berry. Staphylococci look like a bunch of grapes or berries, when observed under a bright microscope. These gram-positive bacteria are facultative anaerobes (www.tetbookofbacteriology.net).

The Staphylococci normally do not cause infectious diseases when living on the skin or in the nose. Staphylococci are opportunists that cause skin disorders when they gain entry in the body through cuts, wounds or damage to the skin. There are many Staphylococcal infections that affect the nose. The following are among diseases/infections carry by staphylococci aureus: Impetigo, furuncles and carbuncles. Taxonomically, the genus *Staphylococcus* belongs to Bacterial family *Staphylococcaceae*, which includes three lesser known genera, Gamella, Macroccoccus and Salinicoccus. The best known of it's nearby phylogenetic relatives are members of the genus *Bacillus* in the family *Bacillaceae*, which is on the same level as the family *Staphylococcaceae*. The *Listeriaceae* are also a nearby family [8].

Staphylococci are facultative anaerobes that grow by anaerobic respiration or by fermentation that yields principally lactic acid. *S. aureus* can grow at a temperature range of 15°C - 45°C and at NaCl concentration as high as 15% [6]. Staphylococci are non-motile, non-spore forming and catalase positive. Their cell wall contains peptidoglycan and teichoic acid [3]. *Staphylococcus aureus* often produce golden yellow colonies, often with hemolysis when grown on blood agar plates [4] More than 90% of *S. aureus* are resistant to the beta-lactamase- resistant penicillin such as methicillin, Nafcillin and Oxacillin, by virtue of changes in the penicillin-binding proteins in their cell membranes. These strains are commonly known as Methicillin resistant *S. aureus* (MRSA) [9].

The following are the important phenotypic characteristics of *Staphylococcus aureus*: -

- Gram-positive, cluster-forming coccus
- Non-motile, non-spore forming facultative anaerobe
- Fermentation of glucose produces mainly lactic acid
- Ferments mannitol in mannitol salt agar (MSA) (distinguishes from *S. epidermis*)
- Catalase positive and coagulase positive
- Golden yellow colony on agar
- Normal flora of human found on nasal passages, skin and mucous membranes
- Pathogen of humans causes a wide range of suppurative infections, as well as food poisoning and toxic shock syndrome.

Penicillins are classified as β -lactam drugs because of their unique four-member lactam ring. They share features of chemistry, mechanism of action, pharmacological and clinical effects, and immunologic characteristics with cephalosporins, monobactams, carbapenems and β -lactam compounds [10].

All penicillin's have the basic structure of a thiazolidine ring attached to a β -lactam ring that carries a secondary amino group (RNH). Substituents (R) can be attached to the amino group. Structural integrity of the 6-amino penicillin acid nucleus is essential for the biologic activity of these compounds. If the β -lactam ring is enzymatically cleaved by β -lactamases, the resulting product, penicilloic acid, that lacks antibacterial activity.

The aim of this study is to isolate and analyze *Staphylococcus aureus* obtained from the anterior nares of female students (at undergraduate and postgraduate levels) at the Obafemi Awolowo University, Ile-Ife campus, Osun state, Nigeria. The study population consisted of forty randomly selected apparently healthy female students. The study was conducted between August to October 2010.

1.2. List of Materials Used

a. Glass wares

The glass wares used were conical flasks, cover slips, beakers, disposable petri-dishes, glass rods, glass slides, measuring cylinder, pipette, test tubes, and glass test tubes.

b. Pieces of Equipment employed

Autoclave, Bunsen burner, light microscope, thermostat, centrifuge, cell colony counter, hot plate, hot air oven, weighing balance.

c. Reagents

Alcohol (95%), crystal violet, distilled water, gram's iodine, human plasma, hydrogen peroxide, immersion oil and safranin.

d. Media

Mannitol salt agar (MSA), DNase Agar, Nutrient broth, normal saline, peptone water

e. Other materials

Marker, inoculating needle, inoculating loop, sterile swab sticks, paper tape, Aluminum foil, cotton wool, vials, test tube rack, forceps, disinfectant, Antibiotic disc.

2. Methods

2.1. Sterilization of Materials

This was a process whereby microorganisms were inactivated, killed or removed from a material. This was done to avoid contamination. The procedure involved the use of an autoclave with steam under pressure. Materials were sterilized in the autoclave at 121°C for 15 minutes.

2.2. Collection of Samples

Nasal swab samples were obtained by using sterile cotton-tipped applicators which were moistened with physiological normal saline. Both the left and the right anterior nares were swabbed by rubbing the cotton-tipped applicators around the inside of each nostril while applying an even pressure and rotating the swab without interruption. Each swab was placed back into the sample container and sealed. The swabs were then labeled and transported to the laboratory immediately for bacteriological analysis.

3. Process

3.1. Sterilization of Other Materials

Materials such as forceps, plastic containers were sterilized using 95°C ethanol. The hands of the researchers and environment where the work was carried out were also disinfected using 95% ethanol.

3.2. Preparation and Sterilization of Media

The media used for this study were Mannitol Salt agar (MSA), Mueller Hinton agar (MHA), Nutrient Broth (NB) and Nutrient (NA) Agar. All the media were prepared according to the manufacturers' specifications. Each medium was weighed and poured into measuring cylinder containing distilled water of required volume after which the medium was heated on hot plate to properly dissolve and sterilized by autoclaving at 121°C for 15 minutes.

3.2.1. Composition and Preparation of Media Used in this Study

i. NORMAL SALINE: This medium was used for the collection and transportation of specimens from the source into the medium used to culture.

Composition: 0.85 g NaCl in 100ml water.

Preparation: 0.85g of NaCl powder was weighed into 100ml of distilled water, shaken to dissolve, then poured into test tubes and then sterilized at 121°C for 15 minutes in an autoclave. Then it was left to cool down.

ii. NUTRIENT BROTH: This medium was used for culturing bacteria and more fastidious organisms.

Composition:

| | |
|---------------|------------------|
| D- Glucose | 1.0g/l |
| Yeast extract | 2.0g/l |
| Peptone | 5.0g/l |
| NaCl | 5.0g/l |
| Final pH= | 7.5± 0.2 at 37°C |

Preparation: 13g of nutrient broth powder was weighed into 1 liter of distilled water. It was swirled to dissolve and then poured into test tubes and sterilized at 121°C for 15 minutes.

iii. MANITOL SALT AGAR: This is a selective medium used for the isolation of Staphylococci.

Composition:

| | |
|--------------------------------|------------------|
| Peptic digest of animal tissue | 5g/l |
| Pancreatic digest of casein | 5g/l |
| Beef extract | 1g/l |
| D-Mannitol | 10g/l |
| Sodium Chloride | 75g/l |
| Phenol red | 0.025g/l |
| Agar | 15g/l |
| Final pH= | 7.4 ±0.2 at 37°C |

Preparation: 108g of MSA powder was weighed into 1 liter of distilled water, heated to dissolve and then sterilized at 121°C for 15 minutes. It was left to cool at 45°C, poured into Petri dishes and allowed to set.

iv. NUTRIENT AGAR: This is a general-purpose medium used for the cultivation of bacteria.

Composition:

| | |
|-----------------|-----------------|
| Meat extract | 1g/l |
| Yeast extract | 2g/l |
| Peptone | 5g/l |
| Sodium chloride | 5g/l |
| Agar | 15g/l |
| Final pH= | 7.4±0.2 at 37°C |

Preparation: 28g of nutrient agar was weighed into 1 liter of distilled water, heated to dissolve, sterilized at 121°C for 15 minutes. It was then allowed to cool to 45°C, poured into petri dishes and allowed to set.

3.3. Isolation or Organisms

Each sample obtained on moistened sterile cotton-tipped applicator from the anterior nares was suspended in

5ml of fresh prepared nutrient broth in a test tube. The culture was then incubated at 37°C for 24 hours. Thereafter, the inoculum was streaked on MSA plates and incubated at 37°C for 48 hours. A discrete, yellow, convex, raised colony was picked from the MSA plate using a sterile inoculating needle and further sub-cultured into nutrient broth and then on nutrient agar for further identification.

3.4. Identification Tests

3.4.1. Gram Stain

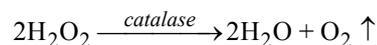
This test was used to distinguish between Gram-positive and Gram-negative organism. About 2-3 discrete colonies of the culture were picked using a sterile inoculating loop and a thin smear was prepared on a clean slide. It was heat fixed. A drop of crystal violet was added to the smear for 1 minute and washed off with water. It was then flooded with Gram's iodine (a mordant) for 1 minute and washed with water. The stain was decolorized with 95% alcohol. A secondary stain (carbon fuchsin) was added for 30 seconds and washed with water. The stain was washed and dried and observed under oil objective after the addition of oil immersion.

Result:

Gram positive organisms stained purple because they retained the purple color of the crystal violet-iodine complex. Gram negative organisms stained pink as they were decolorized by the alcohol treatment and therefore retained the color of the carbon fuchsin which served as the counter stain.

3.4.2. Catalase Test

This was used to differentiate staphylococci from streptococci which are both gram positive [2]. A drop of 3% hydrogen peroxide was placed on a clean glass slide and a colony of the pure isolate from the culture sample was added to it. Staphylococci showed effervescence immediately by converting hydrogen peroxide to oxygen and water.



3.4.3. Coagulase Test

It detected the presence of coagulase. Coagulase causes plasma to clot, it is an important test used to differentiate between *S. aureus* (+) from *S. epidermis* (-) [2].

0.5ml of human plasma was added to a test tube containing 0.5 ml of an 18-24 hr. old broth culture of the test culture. The tube was incubated at 37°C for 24 hours and examined after 4 hours and 24 hours. The presence of clot or coagulation in the tube showed a positive coagulase result, while the absence of clot or coagulation indicated a negative result.

3.4.4. DNase Test

The DNase agar was prepared, sterilized and poured aseptically into petri dishes and allowed to dry and set. A sterile inoculating loop was used to streak fresh culture from the nutrient agar on the DNase agar which was incubated at 37°C for 24 hours. After incubation, 1N of HCL was poured on the plate and after 2-3 minutes,

observations were made. The presence of a clear zone indicated a DNase positive result and its absence indicated a DNase negative result.

4. Results

Out of 40 samples collected from the anterior nares of female students in Obafemi Awolowo Campus, 9 *Staphylococcus aureus* isolates were obtained. All isolates were gram-positive, catalase positive, coagulase positive and all tested positive to DNase.

Table 1. Morphological and biological characteristic of the isolates

| STRAIN CODE | GRAM REACTION | SHAPE | GROWTH ON MSA | CATALASE | COAGULASE | DNase |
|-------------|---------------|-------|---------------|----------|-----------|-------|
| UNS 6 | +ve | Cocci | +ve | +ve | +ve | +ve |
| UNS 9 | +ve | Cocci | +ve | +ve | +ve | +ve |
| UNS 14 | +ve | Cocci | +ve | +ve | +ve | +ve |
| UNS 17 | +ve | Cocci | +ve | +ve | +ve | +ve |
| UNS 41 | +ve | Cocci | +ve | +ve | +ve | +ve |
| UNS 50 | +ve | Cocci | +ve | +ve | +ve | +ve |
| UNS 89 | +ve | Cocci | +ve | +ve | +ve | +ve |
| UNS 103 | +ve | Cocci | +ve | +ve | +ve | +ve |
| UNS 105 | +ve | Cocci | +ve | +ve | +ve | +ve |

5. Discussion and Conclusion

Our results showed the presence of *Staphylococcus aureus* in the nasal nares of participants. Furthermore, the study showed that the human nose is a reservoir for diverse group of microorganisms including *S. aureus* infection. Other investigators have reported that *Staphylococcus aureus* is the major risk factor for the development of infection in certain groups of individuals [11]. *Staphylococcus aureus* is a common bacterium. Most humans carry it in their noses or on skin. But in the 1950's,

less than 10 years after penicillin was made, *Staphylococcus aureus* has become resistant to penicillin that healthy individuals attending hospitals developed infections and diseases. In order to avoid contamination and infection, individuals should practice good and proper hygiene, educated not to abuse antibiotic to which to *Staphylococcus aureus* is resistant, such the β -lantams such as penicillin's and cephalosporin [5,7]. Furthermore, there should be policies guiding the use of drugs and management which will have a great impact on communities.

Appendix

Domain: Bacteria
 Kingdom: Eubacteria
 Phylum: Firmicutes
 Class: Bacilli
 Order: Bacillales
 Family: *Staphylococcaceae*
 Genus: *Staphylococcus*
 Species: *Staphylococcus aureus*

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