

RESEARCH ARTICLE

# A Study on the Susceptibility of *Staphylococcus aureus* From the Nasal Samples of West African Dwarf Goats to Cefoxitin and Trimethoprim

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## Abstract

Goats are important in the livestock economy as they are good sources of protein and income for the rural poor. However, their nasal cavities could serve as reservoirs for *Staphylococcus aureus*, an opportunistic pathogen in human and animal infections. This study investigated the antibiotic susceptibility of *S. aureus* from the nasal samples of the West African Dwarf (WAD) goats in Osogbo, Nigeria to trimethoprim and cefoxitin. Isolates were identified using standard microbiological procedures (Gram staining, catalase, coagulase, DNase and oxidase tests) and the disk diffusion technique was used to perform the antibiotic susceptibility testing. A total of 37 staphylococcal isolates were obtained out of which 14 were identified as *S. aureus*. Antibiotic susceptibility testing showed that 36% (5/14) of the isolates were resistant to both trimethoprim and cefoxitin. The presence of *S. aureus* in the nasal cavity of goats is a potential risk factor for the occurrence of staphylococcal infections in the goat thereby causing economic loss. Furthermore, the occurrence of cefoxitin (methicillin) resistant *S. aureus* in goats is of concern as they could be transmitted to goat handlers and sellers.

**Keywords:** Susceptibility, *Staphylococcus aureus*, Nasal Samples, West African Dwarf Goats

## 1. Introduction

### 1.1 Nasal Cavity of Goat

Goats have gained importance in the Nigerian live stock economy as a result of their remarkable adaptability to adverse environmental conditions (Peacock, 2005). They are good source of protein and income for the rural poor, especially women and children and also a good source of foreign exchange earnings (Ojango *et al.*, 2016). Hence, increase in goat production is needed to maintain food security and increase earnings (Diallo, 2006). In spite of the fact that most households keep goats, their production is not well developed because of factors such as inadequate nutrition, poor management and prevailing diseases.

In Africa, few studies have been conducted on the bacterial characterization of then as cavity of goats (Rahimi *et al.*, 2015; Khalifa *et al.*, 2015; Abdel-Moein and Zaher, 2019) and indicated that it is composed of various species of bacteria. Notable among the mare genus *Staphylococcus*, *Propioni bacteria*, *Enterobacter*, *Klebsiella*, *Corynebacteria*, and *Protovella*. *Staphylococcus aureus* is ubiquitous and have been found to have pathogenic potential on its host (Hildebrandt, 2015). About 25% of the goats' nostrils are a symptomatically and permanently colonized by *S. aureus* (Rahimi *et al.*, 2015). They are widely distributed in nature and are found in a wide variety of mammals and birds as well as most surfaces (Okafor *et al.*, 2011).

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## 1.2 Staphylococci

The name *Staphylococcus* was coined from a Greek word “*staphyle*”, which means bunch of grape and “*kokkos*” meaning berry. They are Gram positive and facultative anaerobic bacteria characterized by individual cocci which divides in more than one planet of grape-like clusters with diameter of 0.5 - 1.5µm (Katayama *et al.*, 2013).

Taxonomically, *Staphylococcus* belongs to the kingdom bacteria, phylum Firmicutes, class Bacilli, order Bacillales, family Staphylococceae, and genus *Staphylococcus*. The genus *Staphylococcus* has about 52 species and 28 subspecies (Edgar, 2018). *Staphylococci* grow by aerobic respiration or by fermentation. The nutritional requirements of most species are relatively complex but generally require an organic source of nitrogen supplied by five to twelve essential amino acids such as valine, arginine and B vitamins, thiamine and nicotinamide (Plata *et al.*, 2009; Kalyani and Saraswathy, 2014).

*Staphylococci* show resistance to heat and tolerance to high salt concentration (Costa *et al.*, 2013). Members of this genus are catalase positive and oxidase negative, differentiating them from the catalase negative streptococci which have a different cell wall composition when compared to the *Staphylococcus* (Plata *et al.*, 2009). They are broadly divided into two main categories of clinical importance: *Staphylococcus aureus* which is coagulase positive, and a heterogeneous group of staphylococci that gives a negative reaction with coagulase test.

## 1.3 Staphylococcus Aureus

*Staphylococcus aureus* appears in grape-like clusters when viewed under a microscope and has large, round, golden-yellow colonies often with haemolysis when grown on blood agar plate (Ray and Ryan, 2004; Winn *et al.*, 2006). *S. aureus* is catalase positive and produces DNase which are extracellular endonucleases that cleaves DNA and release free nucleotides and phosphates (Abdul-Aziz *et al.*, 2015).

## 1.4 Nasal Carriage of S. Aureus in Goats

*S. aureus* is widely distributed in nature, ubiquitous and form small components of the soil microflora (Madigan *et al.*, 2005). Khalifa *et al.* (2015) reported the occurrence of enter toxigenic *S. aureus* from the nasal cavities of goats in Iran (Khalifa *et al.*, 2015). According to a study on *S. aureus* occurrence in then as a cavity of goats by Rahimi *et al.* (2015) in Iran,

25% nasal colonization was reported. The finding agrees with earlier reports made in Tunisia for the same species of goat (Nemeghaire *et al.*, 2014).

Transmission occurs by direct contact with a colonized carrier or contact with excretions such as saliva or aerosols released during sneezing and coughing (El-Jackee *et al.*, 2008). Close human-animal contact occurs during pasture cleaning, feeding or petting the animals. Some farms breed their own goats and use their meat or milk, either for direct consumption or to make cheese (Schilling *et al.*, 2012). It has been widely accepted that small ruminants play a role in the transmission of zoonotic diseases, such as salmonellosis, infections with *Staphylococcus* and many other pathogens (Eriksson *et al.*, 2013).

Since *Staphylococcus aureus* strains come from several sources and many animals are healthy carriers, preventing the spread of infection is a difficult task during the critical period of lambing and kidding, when many animals are in close proximity in a small area (Klimešová *et al.*, 2017). *S. aureus* also cause mastitis in animal species such as cattle, sheep, goats, and horses. It also causes dermatitis in sheep and goats as well as botryomycosis in pigs and horses (Foster, 2012).

## 1.5 Colonization and Pathogenicity

*S. aureus* are potential pathogens which often colonize the skin and mucosal surfaces of healthy animals and humans (Brown *et al.*, 2014). When conditions are favourable, they produce a variety of enzymes which are believed to play a vital role in initiating infections (Kong *et al.*, 2016). These enzymes include nucleases which act on extracellular DNA and RNA, lipases which promote the survival of the bacteria on the skin and hyaluronidase which is a spreading factor that hydrolyses hyaluronic acid. Hyaluronic acid is present in the intracellular ground substance of connective tissue (Udora & Adeniran, 2025).

Others enzymes are staphylokinase (fibrinolysin) which dissolves fibrin clots by promoting the conversion of plasminogen to the fibrinolytic enzyme plasmin, and coagulase (a plasma-clotting protein which binds to prothrombin and converts fibrinogen to fibrin) (Abdul-Aziz *et al.*, 2015). The resistance of *S. aureus* to dehydration and heat and its ability to tolerate many common disinfectants are major properties for their survival and transmission (Costa *et al.*, 2013). The basis for *S. aureus* colonization is complex and not completely understood but known to involve the host's contact with *S. aureus*, its ability

to adhere to host's cells and to evade the immune response (Okafor *et al.*, 2011; Kong *et al.*, 2016).

### 1.6 Antibiotics

Antibiotics are a type of antimicrobial drugs which are used in prevention and treatment of bacterial infections (WHO, 2014). The term antibiotic which means "opposing life", based on Greek roots, anti: "against" and biotic: "life", is broadly used to refer to any substance used against microbes. In the usual medical application, antibiotics (such as penicillin) are those produced naturally (by one microorganism fighting another), whereas non-antibiotic antibacterials (such as sulphonamides and antiseptics) are fully synthetic (Harris *et al.*, 2014). However, both classes have the same goal of killing or preventing the growth of bacteria, and both are included in antimicrobial chemotherapy. Antibiotics may be bactericidal whereby bacteria are killed, or bacteria static in which case the growth than reproduction of the bacteria is inhibited and resumed when the chemical is removed (Nemeth *et al.*, 2014).

### 1.7 Resistance to antibiotics by bacteria

Antibiotics have been used for a long time and are frequently prescribed. This wide spread use has resulted in adaption and changing of bacteria target, making the drugs less effective. This is referred to as antibiotic resistance (Bogolubsky *et al.*, 2016). Using antibiotics when they are not needed especially in livestock contributes to antibiotic resistance and unwanted side effects. Resistance in staphylococci was observed in the early 1940's after the discovery of penicillin (Sharkey *et al.*, 2016; Hoffmann, 2017).

### 1.8 Antibiotic Resistance Mechanism

Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections (Marnoor, 2017). The bacteria survive and continue to multiply causing more harm. Bacteria can do this through several mechanisms. Some bacteria develop the ability to neutralize the antibiotic before it can cause harm, others can rapidly pump the antibiotic out, and still others can change the antibiotic targets so it cannot affect the function of the bacteria (Dibah *et al.*, 2014). The four main mechanisms by which bacteria exhibit resistance to antibiotics include:

### 1.9 Drug Inactivation or Modification

Enzymatic deactivation of penicillin G in some

penicillin-resistant bacteria through the production of  $\beta$ -lactamases. Most commonly, the protective enzymes produced by the bacterial cell will add an acetylphosphate group to a specific site on the antibiotic, which will reduce its ability to bind to the bacterial ribosomes and disrupt protein synthesis (Marnoor, 2017).

### 1.10 Alteration of Target or Binding Site

Alteration of penicillin binding protein (PBP)- the binding target site of penicillin in methicillin-resistant *Staphylococcus aureus* (MRSA) and other penicillin resistant bacteria. Another protective mechanism found among bacterial species is ribosomal protection proteins. These proteins protect the bacterial cell from antibiotics that target the cell's ribosomes to inhibit protein synthesis. The mechanism involves the binding of the ribosomal protection proteins to the ribosomes of the bacterial cell, which in turn changes its conformational shape. This allows the ribosomes to continue synthesizing proteins essential to the cell while preventing antibiotics from binding to the ribosome to inhibit protein synthesis (Connell *et al.*, 2013).

### 1.11 Alteration of metabolic pathway

Some sulphonamide-resistant bacteria do not require para-aminobenzoic acid (PABA). This is an important precursor for the synthesis of folic acids and nucleic acids in bacteria inhibited by sulphonamides. Instead, these sulphonamide-resistant bacteria, like mammalian cells, turn to use preformed folic acid (Root *et al.*, 2018).

### 1.12 Reduced Drug Accumulation

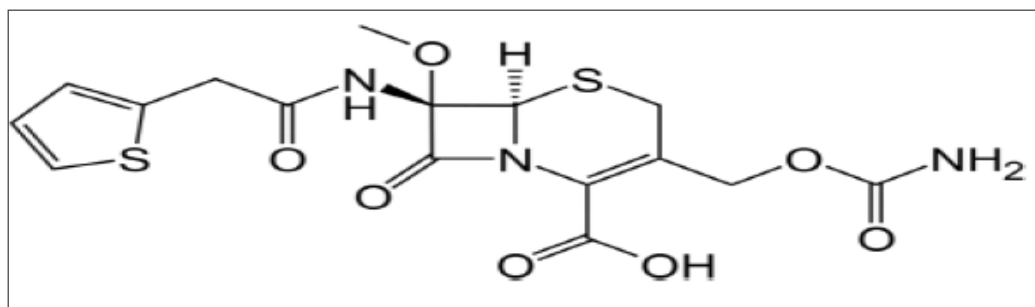
Reduced drug accumulation which occurs by decreasing the drug permeability or increasing active efflux (pumping out) of the drugs across the cell surface (Jang, 2016). These pumps within the cellular membrane of certain bacterial species are used to pump antibiotics out of the cell before they are able to do any damage. They are often activated by a specific substrate associated with an antibiotic (Saxena, 2015).

### 1.13 Cefoxitin

Cefoxitin is a beta-lactam antibiotic derived from cephamycin C, a naturally occurring substance produced by *Streptomyces lactamdurans*. Its resistance to destruction by beta-lactamases results in a broad spectrum of antibacterial activity which includes anaerobic as well as Gram positive and Gram-negative aerobic bacteria, including many resistant to cephalothin and other cephalosporins (Fonzé *et al.*, 2002). The chemical structure of cefoxitin is shown in Figure 1.

Cefoxitin is a chemically modified form of a member of a new family of  $\beta$ -lactam antibiotics. Cefoxitin is a member of the third generation cephalosporins but due to its 7  $\alpha$ -methoxyl group, it possesses important biochemical characteristics not found in other  $\beta$ -lactam antibiotics. The methoxyl group provides the molecule with a remarkable degree of resistance to all

$\beta$ -lactamases. This resistance provides the antibiotic with a broad antibacterial spectrum, including activity against the *Proteus* strains, *Serratia* spp. And *Bacteroides* (Carretto *et al.*, 2018). In addition, cefoxitin is active against many strains of Gram-positive bacteria that have become refractory to the  $\beta$ -lactam antibiotics (Bardal *et al.*, 2011).



**Figure 1.** The structural formula of cefoxitin (Bardal *et al.*, 2011)

#### 1.14 Mechanism of Action

Cefoxitin is a beta lactam antibiotic that interferes with the cell wall synthesis in a bacterium. It binds to the penicillin binding protein (PBP) or transpeptidases and prevents the formation of cross linkages between peptidoglycan layers that make up the cell wall of the bacteria (Fernandes *et al.*, 2005).

#### 1.15 Mechanism of Resistance to Cefoxitin

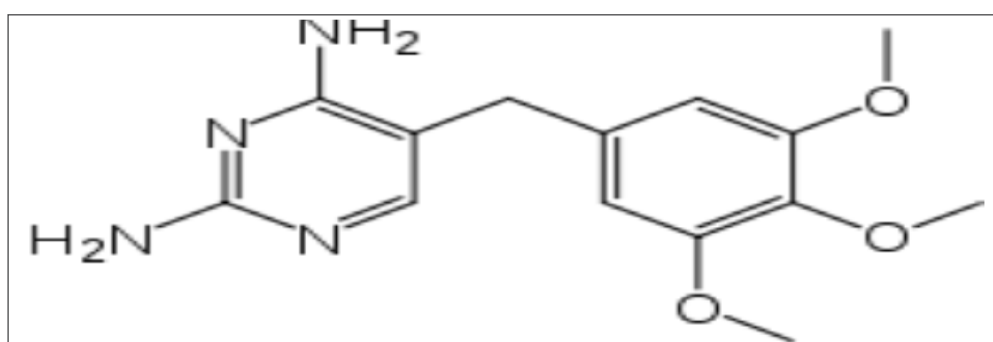
*Staphylococcal* resistance to cefoxitin occurs when an isolate produces an altered penicillin-binding protein, PBP 2a, which is encoded by the *mecA* gene (Dibah *et al.*, 2014). This PBP has a lower binding affinity for penicillin-based antibiotics such as cefoxitin and will continue to cross-link the peptidoglycan layers of the cell wall even in the presence of the beta lactam antibiotics. This results in resistance to this class of antibiotics. Also in the presence of cefoxitin, bacteria that produce a beta-lactamase will increase their production and secretion to cleave the beta-lactam ring (Shaikh *et al.*, 2015). As a cephamycin, cefoxitin is highly resistant to hydrolysis by some beta-lactamases, in part due to the presence of the 7- $\alpha$ -methoxy functional group (Torimiro *et al.*, 2013).

#### 1.16 Trimethoprim

Trimethoprim was discovered in 1962 (May and Hooper, 2017). It was used to treat urinary tract infections, traveller's diarrhoea and middle ear infections in Finland in 1972. It blocks folate metabolism via dihydro folate reductase in some bacteria and results in their death, hence it is bactericidal (Singh *et al.*, 2012). The chemical structure of trimethoprim is shown in Figure 2.

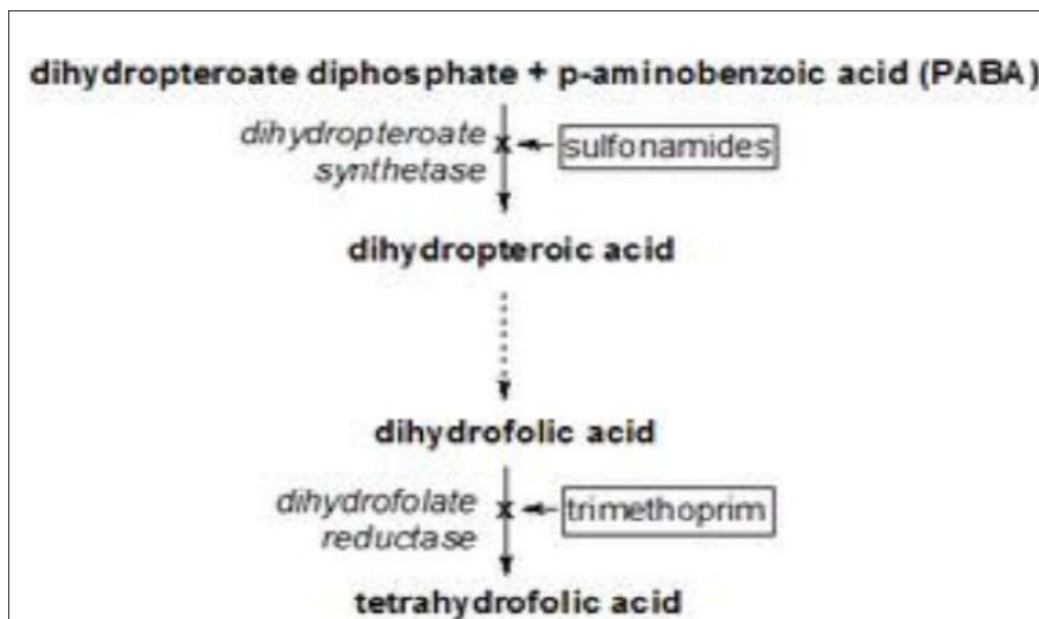
#### 1.17 Mechanism of Action of Trimethoprim

Trimethoprim, 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine, is a synthetic antibiotic that interferes with bacterial biosynthesis of nucleic acid and proteins. It blocks the production of tetrahydro folic acid from dihydro folic acid by binding to and reversibly inhibiting the enzyme dihydrofolate reductase. It has been active against most strains of the bacteria genera *Staphylococcus*, *Enterobacter* and *Klebsiella* (Heaslet *et al.*, 2009). The image shown in Figure 3 reveals the mechanism of action of trimethoprim in tetrahydrofolate synthesis pathway.



**Figure 2.** Structural formula of trimethoprim (Brodgen *et al.*, 2014).





**Figure 3.** Mechanism of action of trimethoprim in tetrahydrofolate synthesis pathway (Heaslet *et al.*, 2009).

### 1.18 Mechanism of Resistance To Trimethoprim

The resistance to trimethoprim result from structural changes that reduce trimethoprim binding to the DHFR enzyme (Vickers *et al.*, 2009). Resistance to trimethoprim is on the rise, yet it is a first line antibiotics in many countries (Otuokere, 2017). Invitro susceptibility of strains of several bacterial species such as *S. aureus* as well as MRSA and coagulase negative *Staphylococcus* indicates potent activity against these pathogens (Abdullah *et al.*, 2014).

### 1.19 Objective of Study

The research objective was to isolate *Staphylococcus aureus* from the nasal cavities of West African Dwarf goats and determine the antibiotic susceptibility of the isolates to trimethoprim and cefoxitin.

## 2. Materials and Method

### 2.1 Materials

#### 2.1.1 Media

Media used include mannitol salt agar, nutrient agar, nutrient broth, Mueller Hinton agar and DNase agar.

#### 2.1.2 Reagents

These include plasma, 70% ethanol, hydrogen peroxide, crystal violet, Gram's iodine, safranin, distilled water, normal saline and immersion oil.

#### 2.1.3 Glassware

Glassware used are beaker, glass slide, test tubes, cover slip, pipettes, conical flasks, glass rod and measuring cylinder.

### 2.1.4 Equipment and apparatus

Weighing balance, oven, electrical microscope, hot plate, colorimeter, stopwatch, hot air oven, refrigerator, incubator, gas cylinder, autoclave, pipette dispenser, Bunsen burner, syringe, cryovials, paper tape, swab sticks, slide clips, cotton wool, inoculating needle, aluminium foil, canister, razor blade, scissors, tray, spatula, foil paper and marker.

## 2.2 Methods

### 2.2.1 Aseptic Techniques

The correct personal protection equipment (PPE) was worn in the laboratory. The work area was kept clean at all times, by swabbing the work bench before and after work with cottonwool soaked in 70% ethanol. Contaminants exist everywhere even in the air, so unnecessary movements, talking and loitering were minimized to avoid contamination. The inoculating loop and needle was sterilized by flaming until red hot using Bunsen burner flame before and after use. The test tubes and conical flasks were flamed each time it was opened to prevent contamination from the air.

### 2.2.2 Preparation of Media

The manufacturer's specifications were strictly followed. Depending on the media required, an appropriate number of solid media was weighed out on a thin foil placed on a weighing balance. The required volume of distilled water was also measured out using a volumetric cylinder. Nutrient broth and other liquid media were dispensed into test tubes and corked with cotton wool before they were sterilized.

### 2.2.3 Sterilization of Media

The media was sterilized using an autoclave. The autoclave works on the principle of moist at sterilization. Moist heat destroys microorganisms by irreversible coagulation and denaturation of their enzymes and natural proteins. Homogenized media were sterilized in an autoclave and allowed to cool to about 45°C, before it was poured into sterile Petri dishes aseptically and allowed to set. The already set agar plates were then inverted before they were put in the oven to test for sterility.

### 2.2.4 Sample Collection

Prior to sample collection, the swab sticks were moistened with sterile normal saline (0.85% NaCl). A total of 102 nasal samples were taken from apparently healthy West African dwarf goat at “Oja-Oba” market, Osogbo (7.8276°N, 4.4834°E), Osun State, Nigeria. The samples were collected by inserting sterile swab sticks into the anterior nares of the goats. The samples were transported immediately to the laboratory for analysis.

### 2.2.5 Isolation and Culturing

Each nasal swab was carefully inserted into labelled test tube containing 5ml nutrient broth. The broth containing inoculum was incubated at 37°C overnight. There after, a loop ful of the broth culture was aseptically streaked on the mannitol salt agar (MSA) and incubated at 37°C for 48 hours. Afterwards, the colonies were examined based on their cultural morphologies and the presumptive staphylococci were picked with an inoculating needle, transferred into nutrient broth and incubated at 37°C overnight. Thereafter, a loop ful of the broth culture was streaked on labelled nutrient agar plates, which was incubated at 37°C for 18-24 hours. Pure colonies of isolates were identified using Gram’s staining and further subjected to other biochemical tests. Pure isolates were stocked in cryogenic vials and stored at- 20°C.

## 2.3 Identification of Isolates Using Microbiological Procedures

### 2.3.1 Gram’s Staining

Gram stain was developed by a Danish Physician in 1884. This differential staining procedure is used to distinguish between Gram positive and Gram-negative bacteria based on distinct and consistent differences in their cell walls. The Gram positive bacteria have a thick layer of peptidoglycan which allows them to retain the primary stain after decolorizing. Gram

negative bacteria have a thin layer of peptidoglycan, so they lose the primary stain after decolorizing and pick up the secondary stain.

#### Procedure

Labelled slides were cleaned with cotton wool soaked with ethanol. A Pasteur pipette was used to place a drop of distilled water on the clean grease free slide. A loop was used to pick about 2-3 distinct colonies of an 18-24-hour old culture on nutrient agar to make a smear. This was air dried and heat fixed. About 1-2 drops of crystal violet solution was added to the heat fixed smear, left for 60 seconds, and washed off under gentle running tap water. The slide was flooded with Gram’s iodine (mordant) for 60 seconds to fix the primary stain, then rinsed with water. The slide was washed with 70% ethanol for about 10-20 seconds and then rinsed immediately under tap water. The counter stain, safranin was added for 30 seconds and washed off with water. Thereafter, the slide was allowed to air dry before viewing under oil immersion objective lens (100X) of alight microscope.

## 2.4 Biochemical Tests

### 2.4.1 Catalase Test

This test is important in the detection of bacteria that produce the enzyme catalase. Catalase is produced by bacteria that live in oxygenated environments to neutralize the toxic form of oxygen metabolites, hydrogen peroxide. The catalase enzyme neutralizes the bactericidal effect of hydrogen peroxide there by protecting them. Catalase mediates the breakdown of hydrogen peroxide to carbon dioxide and water. Catalase test differentiates between bacteria that produces the enzyme catalase such as staphylococci, from non-catalase producing bacteria such as streptococci.

#### Procedure

One drop of hydrogen peroxide solution was placed on a grease-free slide. A sterile inoculating loop was used to pick about 3-4 colonies from an 18-24 hour old culture on nutrient agar and placed on the glass slide. Spontaneous formation of bubbles indicates positive catalase test due to conversion of hydrogen peroxide to oxygen and water. *S.aureus* ATCC 25923 was as used positive control.

### 2.4.2 Coagulase Test

Coagulase test is carried out to detect the production of the enzyme coagulase which converts fibrinogen to fibrin. Coagulase is an enzyme produced by *Staphylococcus aureus* that converts soluble fibrinogen

in plasma to fibrin (insoluble). *Staphylococcus* on the basis of these tests are divided into two groups which are the coagulase positive which forms clumping and the coagulase negative staphylococci in which no clot is formed.

#### *Procedure*

A sterile syringe was used to dispense about 2ml of human plasma into sterile test tubes. About 2-3 colonies of an 18-24 hour old culture on nutrient agar were inoculated into the plasma aseptically. The suspension was incubated at 37°C for 24 hours but checked every 4 hours. Clump formation indicates positive test. *S.aureus* ATCC25923 was used as positive control.

#### **2.4.3 Dnase Test**

This test determines the ability of an enzyme to produce DNase which is an extra cellular endo nuclease that cleaves DNA and releases free nucleotides and phosphates. To detect this enzyme, DNase agar was used to observe the hydrolysis of DNA. This test is used in differentiating *S. aureus* (DNase producers) from other staphylococci that do not produce DNase. Therefore, DNase producing colonies hydrolyse DNA and produce a clear zone around the growth.

#### *Procedure*

A sterile inoculating loop was used to pick two to three colonies from an 18-24 hour old culture on a nutrient agar plate and used to make a single line streak on a DNase agar plate. *S. aureus* ATCC 25923 was used as positive control. The plates were incubated at 37°C for 24 hours. The plates were flooded with 1NHC and left to stand for few minutes to allow the reagent to absorb into the plate. The streak was then observed. Appearance of a clear zone around the streak indicates a positive DNase test.

#### **2.4.4 Oxidase Test**

This test is used to identify bacteria that possess the cytochrome oxidase enzyme. The oxidase enzyme catalyses the oxidation of cytochrome c. The enzyme catalyses the transport of electron between electron donors in a bacterium and a redox dye (tetramethyl-p-phenylene-diamine). Bacteria which contain cytochrome c as part of their respiratory chain are oxidase-positive and turn the reagent blue or purple while those lacking cytochrome c as part of their respiratory chain do not oxidize the reagent. It differentiates *Micrococcus* which gives a positive test from *Staphylococcus* which is oxidase negative.

#### *Procedure*

A sterile inoculating loop was used to pick about 2-3 colonies of 18-24 hour old nutrient agar plate and transferred to an oxidase strip. The area was observed and a colour change to blue or purple indicated positive oxidase test but if the original color of the strip was retained it is oxidase negative. *S. aureus* ATCC 25923 was used as negative control.

#### **2.4.5 Antibiotic Susceptibility Test**

The Kirby Bauer's disk diffusion method was used to perform this test. The susceptibility of the isolates to two antibiotics were tested. The antibiotics used were cefoxitin and trimethoprim.

#### *Procedure*

A pure culture of an 18-24 hour old on nutrient agar was suspended in normal saline and standardized using a colorimeter to 0.5 McFarland standard. A sterile swab stick was inserted inside the inoculum and was used to swab the Mueller Hinton Agar (MHA) evenly over the surface. The plates were rotated while swabbing to ensure even distribution of the inoculum and afterwards the plate was allowed to dry for few seconds. The antibiotics were placed firmly on the surface of the plate using a sterilized forceps. The plates were then inverted and placed in an incubator at 37°C for 24 hours. The results were read by measuring the diameter of the zone of inhibition using a transparent ruler. The results were interpreted as susceptible, intermediate and resistant based on the standard set by the Clinical and Laboratory Standard Institute (CLSI) (CLSI, 2018).

### **3. Results**

#### **3.1 Information on Sample collection**

A total of 102 samples were taken from the anterior nares of apparently healthy African dwarf goats at "Oja-Oba" market, Osogbo, Osun State, Nigeria. All samples were collected within the month of August 2019.

#### **3.2 Identification and Biochemical Tests**

Of the 102 samples collected, 37 staphylococcal isolates were recovered. They were all Gram and catalase positive. From the 37 recovered staphylococcal isolates, 14(37.8%) *Staphylococcus aureus* isolates were identified based on coagulase and DNase results.



### 3.2.1 Antibiotic Susceptibility Pattern

Antibiotic susceptibility testing carried out on the *S.aureus* isolates showed that a total of 5 (35.7%)

were both resistant to trimethoprim and cefoxitin. The summary of the antibiotic susceptibility pattern of the isolates to the antibiotics is shown on Table 1.

**Table 1.** Antibiotic susceptibility of *S.aureus* isolated from nasal swab of West African Dwarf Goat

Antibiotics (disc content)	Susceptible (%)	Intermediate (%)	Resistant (%)
Cefoxitin (30µg)	9(64.3)	—	5(35.7)
Trimethoprim (30µg)	7(50.0)	2(14.3)	5(35.7)

KEY: - = None, % = Percentage

## 4. Conclusion

*Staphylococcus aureus* is a normal flora of animals and humans colonizing the anterior nares of apparently healthy farm animals. However, it is an opportunistic pathogen capable of causing several diseases in livestock (Gharsa *et al.*, 2012). In this study, *S. aureus* isolates were identified from the nasal cavity of West African dwarf goats in Osogbo, Nigeria. This is similar to a report in Egypt where *S.aureus* was recovered from the nasal cavity of healthy ruminants (Daaloul Jedidi *et al.*, 2016). The prevalence of *S.aureus* in the nasal cavity of healthy African dwarf goats may be a predisposing factor for subsequent infections in these animals (Mørk *et al.*, 2010).

Antibiotic susceptibility result showed that 36% of the isolates were resistant to cefoxitin, a surrogate marker for methicillin. Cefoxitin is a second-generation cephalosporin that works by inhibiting transpeptidase enzyme, preventing it from forming cross-links of peptidoglycan (Bardal *et al.*, 2011). Resistance to cefoxitin (methicillin) in *S.aureus* is mainly by the production of an altered penicillin binding protein, PBP2a, encoded by the *mecA* gene, which has lower binding affinity for the antibiotic and continue to cross-link the peptidoglycan can layer of the cell wall (Dibah *et al.*, 2014). In addition, resistance to this antibiotic could be as a result of the production of beta-lactamase, an enzyme that degrades the beta-lactam ring (Bardal *et al.*, 2011).

Furthermore, it was also observed that 36% of the *S.aureus* isolates were resistant to trimethoprim. This could be attributed to the mutation of dihydrofolate reductase in *S.aureus* which confers resistance to trimethoprim (Vickers *et al.*, 2009). Trimethoprim is a diaminopyrimidine antimicrobial agent that exert its antibacterial effect through competitive inhibition of dihydrofolate reductase (DHFR), a key enzyme for folic acid synthesis. The resistance to trimethoprim results from structural changes that reduce trimethoprim binding to the DHFR enzyme (Vickers *et al.*, 2009).

In conclusion, occurrence of trimethoprim and cefoxitin resistant *S.aureus* in goats poses a problem to public health. Therefore, regular hand washing should be practiced among goat handlers and sellers to avoid spread and transmission to humans. Finally, excessive use of antibiotic in livestock management as growth factors and in prophylaxis should be reduced to avoid antibiotic resistance development.

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