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Ecophysiological Profiling and Evaluation of Biofilm Production by *Staphylococcus aureus* Isolated from Meat Abattoir in Ozoro, Delta State, Nigeria

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Abstract

Ecophysiological profiling and evaluation of biofilm production by *Staphylococcus aureus* isolated from Meat Abattoir in Ozoro was carried out to identify and isolate *S. aureus* from the samples from meat contact table in Ozoro and to determine the biofilm forming ability of the *S. aureus* isolated from the samples. A total of eighty (80) samples were collected for analysis from food contact surfaces in Meat markets located in Ozoro town. Samples were collected from meat market at Small market, Big market, Uruto Market, NDC market, and some street meat sellers. A total of 61 *S. aureus* were isolated from food contact surfaces in different meat markets. Twenty-six (26) were strong biofilm producers while 35 were non biofilm producers. 42.6% of the total sixty-one (61) *S. aureus* samples were positive to biofilm formation, while 57.4% were non biofilm producers on Congo red agar. *S. aureus* were more susceptible to Levofloxacin followed by Ofloxacin, with a susceptibility percentage of 83.6% and 81.9% respectively. Also, Augmentin, Cefazidime and Cefuroxine had no effect as an antibiotic against *S. aureus* as their percentage range of susceptibility is 0.0%.

Keywords: *Ecophysiological, Profiling, Staphylococcus aureus, Biofilm, Meat Abattoir*

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Introduction

As opposed to planktonic cells, biofilms in the food industry give bacteria greater resistance to environmental stresses like cleaning, disinfection, and inhibition, allowing them to survive on surfaces and processing equipment (Kostski *et al*., 2012; Laird *et al*., 2012; Bridier *et al*., 2015). Biofilms can form on any kind of technological system surface that comes into touch with food. The presence of harmful bacteria in industrial environments may be linked to the identification of biofilms in the food sector.

There are numerous pathogenic or spoiling bacteria that are affixed to surfaces as planktonic or sessile cells that create a biofilm (Braga *et al*., 2005). According to Costerton *et al*. (1999), biofilms are collections of microbial cells encased in an exopolymer matrix that provides resistance to these microbes. It is well known that bacteria that group together to

form biofilms are more resilient to environmental stressors, such as sanitizers and other antimicrobials, than their planktonic counterparts (Fux *et al*., 2004).
One common bacterial species that is Gram-positive is *Staphylococcus aureus*. About 20–25% of people on Earth have been colonized consistently, while 75–80% have been colonized sporadically or never. Prior research has demonstrated a clear causal relationship between nasal carriage of *S. aureus* and an elevated risk of nosocomial infection. After being transferred to the circulatory system through an epithelial breach, *S. aureus* uses nasal carriage as a staging ground to spread to other parts of the body, where it causes planktonic growth and the upregulation of adhesion factors. The host's innate immune reaction subsequently either eliminates the invasive staphylococci or they adhere to the extracellular matrix proteins and create a biofilm. After that, the cellular physiology rapidly changes to reflect that of a biofilm. *Staphylococcus aureus* continues to garner a lot of attention due to its increasing role in infections related to foreign bodies, its rapid development and display of multiple-antibiotic resistance, and its tendency to progress from an acute infection to one that is persistent, chronic, and recurrent.

Inadequate sanitation procedures in food processing facilities can lead to pathogen contamination of food items, posing a major risk to customers' health (Orogu *et al*., 2017;. Ehiwario *et al*., 2025). Furthermore, bacteria can adhere to surfaces that come into contact with food and form biofilms, where they persist long after cleaning and disinfection, making it challenging to completely eradicate pathogens from food processing environments (Brooks and Flint, 2008; Arekemase *et. al*., 2019). The most prevalent bacterial lifestyle in nature is that of biofilms. In the majority of food business sectors, biofilms are a major issue. An infection with the *Staphylococcus aureus* bacteria is the cause of staphylococcal food poisoning (Case, 2004; Okinedo *et al*., 2024). Food poisoning and other food-borne illnesses are caused by the bacteria *Staphylococcus aureus*, which can be found in food (Foskett *et al*., 2003; Orogu and Ehiwario, 2025). A toxin or poison secreted by bacteria belonging to the staphylococcus group is the cause of staphylococcal food poisoning (Lennox *et al*., 2012). People who consume food that has been incorrectly stored or prepared—especially processed meats, poultry, pastries, and hollandaise sauce—where *Staphylococcus aureus* has proliferated may acquire this food-borne intoxication (Prescott *et al*., 2008).

Both the environment and normal human flora contain S. aureus, which is found on the skin and mucous membranes (mostly the nasal area) of the majority of healthy people (Rasigade and Vandenesch, 2014). Although *S. aureus* often does not infect healthy skin, it can cause a number of potentially dangerous infections if it is let to penetrate the bloodstream or internal tissues (Rasigade and Vandenesch, 2014). Direct contact is usually the source of transmission. On the other hand, certain illnesses are spread by different means (Tong *et al*., 2015).

Staphylococcal food poisoning can be caused by the food-borne bacterium *Staphylococcus aureus* (Okobia and Orogu 2021). According to estimates, staphylolcoccal food poisoning causes 241,188 illnesses, 1,064 hospitalizations, and six fatalities in the United States per year (Scallan *et al*., 2011). The quality and safety of food products can be impacted by *S. aureus*'s ability to stick to and form biofilms on surfaces that come into contact with food. Srey *et al*. (2013) and Marques *et al*. (2007).

Methicillin-resistant *S. aureus* (MRSA) and other *Staphylococcus aureus* strains are known to produce biofilms and to cause substantial morbidity and mortality in humans. Complex genetic variables closely regulate the biofilm development mode of *S. aureus*. Chronic illness results from mostly inefficient host immune responses to persistent biofilm infections. Nonetheless, recent studies have considered biofilm formation to clarify host immunity against infection, which could result in the creation of effective anti-biofilm *S. aureus* treatments.

Exopolysaccharide (PIA/PNAG) often makes up the extracellular matrix of S. aureus biofilms, however staphylococcal biofilms can also contain proteinaceous and extracellular DNA matrix (Boles *et al*., 2010). Blood components or non-cellular elements including mineral crystals, corrosion particles, and clay or silt particles may also be present in the biofilm matrix, depending on the conditions under which the biofilm formed (Donlan, 2002). The irreversible attachment phase is associated with PIA (Szczuka *et al*., 2013). In addition to being mediated by PIA-dependent biofilm formation, *Staphylococcus aureus* can also generate biofilms that are PIA-independent. Biofilm-associated protein (Bap) and Bap-related proteins of *S. aureus* can confer biofilm development independently or PIA production through cell-to-lcell aggregation in the PIA-independent biofilm, despite the significance of the ica gene locus in biofilm development. These proteins are distinguished by their high molecular weight, presence of the bacterial surface, function as a virulence factor, and occasional containment in mobile elements (Lasa and Penades, 2006; Archer *et al*., 2011).

Therefore, the aim of this study was to isolate and identify Staphylococcus aureus from meat contact surfaces in meat markets in Ozoro, Delta State, Nigeria, and to evaluate their biofilm-forming ability and antibiotic susceptibility profiles.

Materials and Methods

Study Area

This research was conducted from Ozoro, Isoko North L.G.A of Delta state in Nigeria. Ozoro is situated along the high way between Ughelli and Kwale town. This location is chosen because it is a fast growing community with high social activities being boosted by the presence of a tertiary institution (Southern Delta University). It is densely populated and their major occupation is farming and trading.

Samples Collection

A total of eighty (80) samples were collected for analysis from food contact surfaces in Meat markets located in Ozoro town. Samples were collected from meat market at Small market, Big market, Uruto Market, NDC market, and some street meat sellers.

Sterilization of Materials
It was ensured that all glassware used was sterilized using the autoclave. Also before and during culturing, the inoculation loop was sterilized by passing it through a Bunsen flame until red hot and allowed to cook before using for inoculation. The work bench was sterilized by cleaning with cotton wool dipped in 70% alcohol.

Materials
The materials used in this study include laboratory coat, gloves weighing balance, cotton wool, slides, sterile universal container, foil paper, incubator, microscope, measuring cylinder (50ml, 250ml, 500ml) , and beakers (50ml, 200ml, 500ml) pipette, petri dishes, test tubes, conical flask, Bunsen burner, nutrient agar, wire loop and water.

Media Used
Mannitol salt agar was used for isolation of the organisms. Nutrient agar for general purpose. DNAse media was used for the DNAse biochemical test. The media used for biofilm determination was Congo red agar.

Method
Sample inoculation
From the food contact surfaces (FCS) of a few meat markets in Ozoro, *S. aureus* strains were identified. Swab sticks soaked in regular saline were used to gather the samples. After being inoculated onto Mannitol salt agar, the samples were incubated for a 24 hours.
Colonies with a defined golden yellow growth was sub cultured on nutrient agar for 24 hours to obtain pure cultures.

Identification of the isolate
Morphological characteristics, Gram staining, and biochemical characterization, such as catalase, coagulase, and DNAse assays, were used to evaluate the isolates and confirm the organisms' identities. Bergey's manual of determinative bacteriology was used to identify the isolates and validate the identification of the bacteria.

Gram staining
A thin smear of each of the pure culture was prepared on clean grease free slides, fixed by passing over gentle flame. Each heat fixed smear was stained by addition of 2 drops of crystal violet solution (primary stain) for 60 seconds and rinsed with water. The smear was flooded with Lugol's iodine for 30 seconds and rinsed with water, decolorized with 70% alcohol for 15 seconds and was rinsed with distilled water. After adding two drops of the secondary stain Safranin for 60 seconds, the area was washed with water and left to air dry. A microscope was used to see the smear with an oil immersion objective lens. Gram positive organisms were purple, whereas gram negative cells were pink or red (Joanne *et al*. 2011).

Biochemical Test
Coagulase test
A drop of sterile distilled water was placed on a clean glass slide. A colony of the test organism was emulsified with the drop of distilled water to make a thick suspension. A loopfull of human plasma was stirred into the suspension on the slide. Clumping visible to the eye within 5-10 seconds indicates positive result (Cheesbrough, 2004).

Catalase test
Using a small sterile applicator stick, a small quantity of the culture was transferred into a drop of freshly prepared 3% hydrogen peroxide solution in a clean slide. Immediate bubble production indicated a positive test and no bubbling indicated a negative test (Cheesbrough, 2004).

DNASE Test
This test determines the ability of an organism that produces DNase. DNase is an extracellular endonuclease that cleave DNA and release free nucleotides and phosphate. To detect these enzymes, DNase agar using no indicators or various indicators (toluidine blue or methyl green) is used to detect the hydrolysis of DNA.

The test organism was inoculated in the DNAse agar (with methyl green indicator) plate with a swab stick. The plate was incubated overnight. Positive test result is recorded if there's development of clear halo around the colony and Negative if no clear zone in the medium. Agar remains green due to no degradation of DNA.

Susceptibility of isolates to antibiotics
Susceptibility testing:
The test was conducted by the disc diffusion method of Bauer *et al.* (2001). The antibiotic discs were spaciously placed on inoculated Mueller-Hinton agar plates Inoculated with the isolates. The inverted plates were incubated aerobically at 37°C for 16 to 18 h. Zones of inhibition, to the nearest millimeter, were interpreted as susceptible, intermediate and resistant based on the interpretative table recommended by the disc manufacturer.

Biofilm formation assay
Phenotype Analysis of Biofilm production
Congo red agar (CRA) plates were used to cultivate the isolates and define their phenotype (Arciola *et al*., 2001). In short, 1 L of blood agar was mixed with 0.8 g of Congo red and 36 g of saccharose to create agar plates, which were then incubated for 24 and 48 hours at 37°C. The S. aureus isolates in the CRA were examined for their macroscopic characteristics. Arciola *et al.* (2005) identified smooth pink colonies as non-producers, crusty black colonies with a dry filamentous appearance as producers of biofilm, and intermediate colony morphology (pink with dark centers resembling bull's eyes) as possible producers of biofilm.

Results

A total of 61 *S. aureus* were isolated from food contact surfaces in different meat markets. Twenty-six (26) samples were strong biofilm producers while 35 were non biofilm producers.

Table 1: The occurrence of *S. aureus* in food contact surfaces from different meat market. This table shows the total number of samples yielding *S. aureus*, the total number of coagulase

negative *Staphylococcus aureus* and the number of samples without growth.

Table 2: Colonial and Biochemical features of the isolates. This table shows the gram reaction and biochemical features of the isolates so as to differentiate between *S. aureus* and other *Staphylococcus species*.

Table 3: Drug susceptibility profile of *S. aureus* isolates from food contact surface. This table shows the antibiotics susceptibility profile of *Staphylococcus aureus*.

Table 4: Biofilm formation by the *S. aureus* isolates (Congo red method). This table shows the number of biofilm forming *S. aureus* and the number of non-formers.

Table 1: Occurrence of *S. aureus* contact surfaces from different meat markets.

Growth on MSA plates	**No of isolates (%)**
No (%) of samples yielding *S. aureus*	61 (61.0)
No of samples with CONs	23 (23.0)
No of samples without growth	39 (39.0)

*****MSA = Mannitol salt agar
CONs = Coagulase Negative *staphylococci*

Table 2: Colonial and Biochemical features of the isolates.

	Isolate 1	Isolate 2
Colonial description	Bright yellow smooth Colonies on MSA plates	milkish white colonies on MSA plates
Gram status	+ve	+ve
Cell arrangement	cocci in clusters	cocci in clusters
Catalase test	+ve	+ve
Coagulase test	+ve	+ve
DNAse test	+ve	-ve
Organism	*Staphylococcus aureus*	CONs

***CONs = Coagulase Negative staphylococci**

Table 3: Drug Susceptibility Profile of *S. aureus* isolates from Food Contact Surfaces.

Antibiotics	No (%) Susceptible	No (%) Intermediate	No (%) Resistant
OFL(5ug)	50(81.9)	8(13.1)	3(4.9)
AUG(30ug)	0(0.0)	0(0.0)	61(100)
CAZ(30ug)	0(0.0)	0(0.0)	61(100)
CRX(30ug)	0(0.0)	0(0.0)	61(100)
GEN(30ug)	42(68.9)	12(19.7)	7(11.4)
CTR(30ug)	9(14.8)	5(8.2)	47(77.0)
ERY(15ug)	12(19.7)	6(9.8)	43(70.5)
CIP(5ug)	48(78.7)	9(14.8)	4(6.6)
ZEM(5ug)	3(4.9)	7(11.4)	51(83.6)
LBC(5ug)	51(83.6)	4(6.6)	6(9.8)

*OFL: Ofloxacin, AUG: Augmentin, CAZ:Cefazidime, CRX:Cefuroxine, GEN: Gentamicin, CTR: Ceftriaxone, ERY: Erythromycin, CIP: Ciprofloxacin, ZEM: Cefepime, LBC: Levofloxacin.

Table 4: Biofilm formation by the *S. aureus* isolates (Congo red method).

Colony appearance on CRA	No of isolates (%)
Pink / red (negative)	35 (57.4)
Black colonies (positive)	26 (42.6)
Total	(61)

***CRA = Congo red agar.**

Discussion

This study examined the applicability of Congo red agar method to identify the biofilm-forming organisms that were isolated from food-contact surfaces in several meat markets in Ozoro.

Biofilm formation is relatively a common phenomenon among many microorganisms, *S. aureus* inclusive. The balance between biofilm type and planktonic growth is influenced by a vast variety of regulatory mechanisms.

On Congo red agar, the study found that 57.4% of the totals (61) *S. aureus* did not create biofilm, whereas only 42.6% of them did. De Silva (2002) demonstrated the apparent inefficiency of biofilm production. Many infections are known to be caused by *S. aureus* heightened susceptibility to various antibiotics and chemotherapeutic treatments. The resident food microflora is relatively dynamic and susceptibility and resistance patterns of microorganisms isolated from food environment sample can vary significantly (Kochman, 2005).

The study demonstrated that *S. aureus* from food environment showed high susceptibility towards majority of tested antibiotics and susceptibility pattern only slightly varied among investigate *S. aureus* isolates. This result is in line with the report of Okobia and Okinedo (Okobia and Orogu 2021; Okinedo *et al*., 2024) who isolated high percentage of *S. aureus in food*. Many studies showed that resistance of *S. aureus* to selected antibiotics can vary among strains within a broad range (Kochman, 2005; Michiniwska-swincowand szychlinska, 2001; Tunger *et al.*, 2001). *S. aureus* were more susceptible to Levofloxacin followed by Ofloxacin, with a susceptibility percentage of 83.6% and 81.9% respectively. Also Augmentin, Cefazidime and Cefuroxine had no effect as an antibiotic against *S. aureus* as their percentage range of susceptibility is 0.0%. This result is in line with the works of Robert (Robert *et al.*, 2014). Although the food environment is always protected and treated, but many biofilm forming organisms still withstand this treatment with antimicrobial agents.

Conclusion

This study demonstrated *S. aureus*'s capacity to produce biofilms after being isolated from meat market surfaces that come into touch with food. When food contact surfaces are contaminated by pathogens that are resistant to hygiene treatments, biofilm formation can result in both public health issues and financial losses. Even though the examined strains of *S. aureus* were responsive to the tested chemotherapeutics, a significant portion of them formed biofilms, which could lead to issues with chemotherapy for infections, especially when it comes to dose determination. Given this, it is clear that knowledge of the bacterial species that are prevalent in the food environment, their patterns of susceptibility and resistance, and their capacity to form biofilms is essential for both physicians and healthcare professionals in developing effective antibacterial treatments and implementing infection prevention and control strategies.

To treat illnesses linked to biofilms, additional research is required on anti-biofilm medications that can be used in conjunction with antibiotics. Checking the spread of these mobile genetic elements should be a priority. Given the rapid rise in drug resistance in bacteria, more regular surveillance and monitoring research should be carried out to inform doctors about the best empirical treatment for the majority of biofilm infections.

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