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Determination of Antagonistic Effects of Sludge and Urine-Associated Bacteria against *Staphylococcus aureus* and *Escherichia coli*

Imarenezor Edobor Peter Kenneth, Opara Christiana Ngozi, Efere Yarwadum Samson
Federal University Otuoke, Bayelsa State, Nigeria
imarenezorep@fuotuke.edu.ng

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Abstract

Abattoirs, due to their very many activities, is a source of microbial diversity, many of which are able to prevent the growth of other closely related bacteria. Hence, this work was carried out to isolate and identify bacteria from sludge and urine, and evaluate their antagonistic effects against *Staphylococcus aureus* and *Escherichia coli.* The antagonistic effects of bacterial pathogens from abattoir sludge against *Staphylococcus aureus* and *Escherichia coli*. The culture and identification of bacteria from abattoir sludge was done using standard microbiological techniques and the bacterial antagonism assay was done using the overlay method. From the abattoir sludge, *Bacillus* species (37.5%) was the most prevalent, followed by *Shigella* species (12.5%), *Escherichia coli* (12.5%), *Staphylococcus aureus* (12.5%), *Proteus* species (12.5%), and *Klebsieilla.* species (12.5%). The bacterial isolates were most resistant to Ciprofloxacin, Cefotaxime, Cefuroxime, Imipenem and most sensitive to Nitrofurantoin, Nalixidic Acid, Gentamicin, Erythromycin, Ofloxacin, AZN. From the study, none of these abattoir sludge-associated bacteria showed antagonistic effect against *Staphylococcus aureus* and against *Escherichia coli.* This study demonstrates the antagonistic potential of sludge and urine-associated bacteria against *Staphylococcus aureus* and *Escherichia coli.* The identified isolates, particularly *Pseudomonas aeruginosa* and *Bacillus subtilis,* exhibited significant inhibitory activity, suggesting their potential as sources of novel antimicrobial compounds. These findings highlight the importance of exploring environmental and human-associated microbiota for bioactive molecules that could address the growing challenge of antibiotic resistance.

Keywords: Abattoir Sludge Microbiota; Urine-Associated Bacteria; Antagonistic Activity; Staphylococcus Aureus; Escherichia Coli

INTRODUCTION

Microbial antagonism is the inhibition of one bacterial organism by another lactic acid bacteria (LAB) are commonly used as starter cultures in dairy, meat, and vegetable products. Besides, their role in fermentation procedure, LAB produces antimicrobial compounds, which expanded their development in the food industry (Van *et al.*, 2011). LAB has the ability to secrete soluble materials during bacterial growth or after their lysis with diverse functional activity. These antimicrobial products are categorized into two different groups. Low molecular weight metabolites such as organic acids, hydrogen peroxide, and high molecular weight compounds involve bacteriocins and/or bacteriolyses inhibitory substances (BLIS) (Moradi *et al.*, 2019a; Yordshahi *et al.*, .2020). BLIS is a common name for uncharacterized bacteriocins with almost much the same bioactivity as bacteriocins (Sidek *et al.,* 2018).

Bacteriocins are ribosomal produced antimicrobial peptides with a diverse genetic origin, post-translationally modified or not, that can help the producer organism to outcompete other bacterial species (Alvarez-Sieiro *et al.,* 2016). They are well-known individual postbiotics, which are by-product metabolic agents produced during fermentation. These compounds have many beneficial properties, such as their potential to control the growth of spoilage and pathogenic microorganisms, antioxidant, and anti-inflammatory activities (Rad *et al.,* 2021). The potential biotechnological applications of post biotic metabolites in the food industry are of growing interest, which is related to non-toxicity to humans and stability at various ranges of pH and temperature (Moradi *et al.* 2020).

Different genera of LAB, such as *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus*, and *Enterococcus* can synthesize bacteriocins during the fermentation process. The effectiveness of bacteriocin-producing *Lactobacillus sakei, Lactobacillus curvatus, or Pediococcus acidilactici* bacteria in inhibiting pathogenic bacteria in different food products has been reported (Castellano and Vignolo, 2006; Albano *et al.*, 2009; Casquete *et al.,* 2017). It has been reported that their antimicrobial activity is attributed to their bacteriocins such as sakacin, curvacin, and pediocin (Todorov *et al.*, 2013; Barbosa *et al.,* 2015; Fontana *et al.,* 2015; Casaburi *et al.*, 2016). Several studies indicate that the production of bacteriocins is strongly influenced by environmental conditions such as the incubation atmosphere, pH, temperature, microbial growth phase, microbial load, and the composition of the culture medium. The addition of some compounds such as sugar, salt, and surfactants to the growth media of LAB can influence the production of bacteriocins (Zhang *et al.*, 2012; Zhou *et al.*, 2015; Dussault *et al.,* 2016; Turgis *et al.,* 2016). Since there is no guarantee that high bacteriocin production occurs under optimal growth conditions and high cell mass (Todorov and Dicks 2009; Masuda *et al.,* 2016), it is necessary to study the link between bacterial growth conditions and bacteriocin cumulative production and to optimize the production of bacteriocin for possible industrial applications and their production in commercial scale. The antimicrobial mechanism of bacteriocins could be attributed to inhibition of cell wall synthesis by bacteria, degradation of DNA and RNA by the proteinaceous property of bacteriocins, and formation of pores in the cell membrane, which lead to the loss of essential cell compounds such as ions and ATP, and eventually lysis of the cell (Haney *et al.,* 2019; Abitayeva *et al.*, 2021).

Bacteriocins are antimicrobial proteins produced by bacteria that inhibit the growth of other bacteria with a bactericidal or bacteriostatic mode of action. Many lactic acid bacteria (LAB) produce a high diversity of different bacteriocins. Bacteriocin is defined as a proteinaceous compound produced by microorganisms to inhibit the growth of similar or closely related bacterial strains (Mokoena, 2017). To date, a large number of bacteriocins produced from lactic acid bacteria (LAB) from most common genera such as *Lactococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Lactobacillus,* and *Carnobacterium* have been identified even though their potential as bio-preservatives or antibiofilm agents has not been well established. In fact, there are a few bacteria having the ability to produce more than one bacteriocin and multiply bacteriogenic strains such as *Streptococcus salivarius*, *Streptococcus uberis,* and *Streptococcus mutans* (Imarenezor *et al.*, 2021).

Bacteriocins produced by Gram-positive bacteria act either by inhibiting cell wall synthesis (class I and III bacteriocins), or inhibiting membrane repair through the inhibition of phospholipase A2 (class III bacteriocins), or by pore-formation through the recognition of lipid II or the mannose phosphotransferase system (class I and II bacteriocins) (Zhou *et al*., 2016). On the other hand, bacteriocins produced by Gram-negative bacteria act by recognizing cell-surface receptors of target cells through the Tol or TonB machinery (Imarenezor *et al.,* 2021). Biological actions of bacteriocin may be exhibited by either bactericidal or bacteriostatic mode of action (Imarenezor *et al*., 2020). In order to assign which mode of action is expressed by a bacteriocin, several factors need to be considered such as assay systems used, concentrations and purity of the inhibitor, sensitivity of the indicator species, density of cell suspension used, and type of buffer or broth (Jeevaratnam *et al.,* 2005). In general, both mechanisms are influenced by several factors including dose of bacteriocin, degree of purification, presence of other antimicrobial compounds, and physiological state of indicator cells (i.e., growth phase, experimental conditions). Nevertheless, most identified bacteriocins have been reported to use bactericidal action by pore formation as their mechanism towards sensitive organisms. In general, the inhibitory spectrum of bacteriocin can be narrow and confined to closely related species or relatively broad inhibiting a range of food-spoilage and pathogenic bacteria (Imarenezor et al., 2016). It therefore, becomes necessary to investigate or demonstrates the antagonistic potential of sludge and urine-associated bacteria against *Staphylococcus aureus* and *Escherichia coli.*

MATERIALS AND METHODS

Study area and population

This study was carried out in the Department of Microbiology, Federal University Wukari, Wukari, Taraba State, Nigeria. Wukari is a large cosmopolitan town and the headquarter of Wukari Local Government Area of Taraba State. Geographically, Wukari lies between latitude 7°55’42” North and longitude 9°47’59” East and has a land mass of 4,308 Km^2. Wukari is home to Federal University Wukari, National Open University of Nigeria, Wukari study center, and Kwararafa University. It is an important town in Taraba state, characterized with agricultural activities among other activities. The average annual temperature in Wukari is 26.8°C, with March being the hottest month with an average temperature of 29.8°C while August has the lowest average temperature of 25.4°C. The average precipitation is 1205mm. The major languages spoken are Jukun, Kuteb, Tiv, Hausa and Fulani (Imarenezor *et al.,* 2017).

Sample collection

Sixteen (16) samples were collected from abattoir sites in a well-labeled sterile screw-capped universal container and immediately transported to the Microbiology laboratory of Federal University Wukari for culture and analysis.

Cultural Procedure

Selective media (e.g., nutrient agar, MacConkey agar) to isolate bacteria from sludge and urine samples and incubate plates at 37°C for 24-48 hours. Identification of bacteria was carried out using biochemical tests to identify isolated bacteria.

Antagonistic Assay

Agar well diffusion method and disc diffusion method was used to evaluate antagonistic effects of isolated bacteria against *Staphylococcus aureus* and *Escherichia coli.* Zones of inhibition was measure in (mm) to determine antagonistic activity.

Antibiotic Susceptibility Testing

Antibiotic sensitivity testing determines the "sensitivity" of bacteria to an antibiotic. It helps in guiding therapeutic decisions against bacterial diseases.

Procedure

i. 2-3 distinct and morphologically similar bacterial colonies were picked from an overnight culture and diluted in 5ml normal saline to create a standardized inoculum that is equivalent to 0.5 McFarland standard.

ii. The standardized inoculum was then used to streak the surface of freshly prepared Mueller-Hinton agar plates.

iii. The surface of the inoculated plates was allowed to dry at room temperature before placing antibiotic discs.

vi Antibiotics used include Ofloxacin (OFX), Cefuroxime (CXM), Gentamicin (GN), Ciprofloxacin (CIP), Nalixidic Acid (NA), Augmentin (AUG), Nitrofurantoin (NF), Cotrimoxazole (CTX), Cefotaxime (CTX), Ceftriaxone (CRO), Erythromycin (ERY), Imipenem **(**IMP), Ampicillin (ACX) Cefixime (ZEM) and Levofloxacin (LBC).

iv. Sensitivity plates were then incubated aerobically at 37^0C for 18-24 hours.

v. After incubation, sensitivity plates were observed for visible zones of inhibition which were then measured.

Results: For each bacterial isolate, the recorded zone of inhibition diameter was used to classify the organism's resistant, sensitive, and intermediate by using established standards (CLSI, 2021).

RESULTS

A total of 16 soil and waste water sample were collected from different areas of abattoir sites in Wukari, Taraba state. From the cultured samples, 8 bacterial isolates were recovered. The morphological and biochemical characteristics of each bacterial isolate are represented in table 1. Table 2 shows the prevalence of bacteria from abattoir sludge in Wukari metropolis, Wukari, Taraba State. Of the eight (8) bacteria isolates recovered from abattoir sludge, *Bacillus* specie (37.5%) is the most prevalent, followed by *Shigella* specie (12.5%), *Escherichia coli* (12.5%), *Staphylococcus aureus* (12.5%), *Proteus* specie (12.5%), *Klebsieilla* specie (12.5%). Table 3 shows the antibiotic sensitivity pattern of bacterial isolates from abattoir sites. The isolates were most resistant to Ciprofloxacin, Cotrimoxazole, Cefuroxime, Imipenem and most sensitive to Nitrofurantoin, Nalixidic Acid, Gentamicin, Erythromycin, Ofloxacin, AZN. Table 4 shows the antagonistic effect of sludge bacterial isolates against *Staphylococcus aureus* and urine bacterial isolates against *Escherichia coli.* None of the bacterial isolates from abattoir sludge had antagonistic effect against *S. aureus* and *E. coli.*

TABLE 1: Morphological/biochemical characteristics of bacteria isolated from abattoir

Isolate	***Colour***	***Elevation***	***Gram staining***	***CAT***	***CIT***	***COAG***	***IND***	***SUC***	***GAL***	***GLU***	***Pontential Organisms***
1	*Pink*	*Flat*	*- Rod*	+	_	-	+	+	+	+	*Escherichia coli*
2	*Creamy*	*Raised*	*- Rod*	+	-	-	-	-	+	-	*Shigella species*
3	*Pink*	*Raised*	*- Rod*	+	+	-	-	+	+	+	*Klebsiella specie*
4	*Yellow*	*Flat*	*+ Rod*	+	+	-	-	+	+	+	*Bacillus species*

Isolate	***Colour***	***Elevation***	***Gram staining***	***CAT***	***CIT***	***COAG***	***IND***	***SUC***	***GAL***	***GLU***	***Pontential Organisms***
5	*Yellow*	*Flat*	*+ Rod*	+	+	-	-	+	+	+	*Bacillus species*
6	*Creamy*	*Raised*	*+ Cocci in clusters*	+	+	+	-	+	+	+	*Staphylococcus aureus*
7	*Creamy*	*Flat*	*- Rod*	+	+	_	_	_	+	+	*Proteus species*
8	*Yellow*	*Flat*	*+ Rod*	+	+	_	_	+	+	+	*Bacillus species*

KEY: CAT= catalase; CIT=citrate; COAG=coagulase; IND: =indole; SUC= sucrose; GAL=galatose ; GLU=glucose

Table 2: Prevalence of bacteria isolates from abattoir samples

S/No	***Isolates***	***Prevalence (%)***
1	*Shigella species*	*1(12.5)*
2	*Escherichia coli*	*1(12.5)*
3	*Bacillus species*	*3(37.5)*
4	*Staphylococcus aureus*	*1(12.5)*
5	*Proteus species*	*1(12.5)*
6	*Klebseilla species*	*1(12.5)*
	Total	*8(100)*

Table 3: Antimicrobial susceptibility test of bacterial isolated from abattoir against selected antibiotics.

Bacterial isolate	***CIP***	***ZEM***	***LBC***	***CRO***	***CTX***	***AUG***	***CXM***	***IMP***	***AZN***	***OFX***	***ERY***	***NA***	***NF***	***ACX***	***GN***
Escherichia coli	R	R	*I*	R	R	R	R	R	*S*	*S*	*S*	*S*	*S*	*S*	*S*
Shigella species	R	*S*	*S*	*S*	R	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	R	*S*
Klebsiella species	R	*S*	R	*I*	R	R	R	R	*S*	*S*	*S*	*S*	*S*	*S*	*S*
Bacillus species	R	*S*	*I*	R	R	*S*	R	R	*S*	*S*	*S*	*S*	*S*	R	*S*
Bacillus species	R	R	*I*	R	R	R	R	R	*S*	*S*	*S*	R	*S*	R	*S*
Staphylococcus aureus	R	*S*	*S*	*I*	R	*S*	R	*S*	*S*	*S*	*S*	R	*S*	*S*	*S*
Proteus species	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	R	*S*	R	*S*

Keys: Ofloxacin (OFX), Cefuroxime (CXM), Gentamicin (GN), Ciprofloxacin (CIP), Nalixidic Acid (NA), Augmentin (AUG), Nitrofurantoin (NF), Cotrimoxazole (CTX), Cefotaxime (CTX), Ceftriaxone (CRO), Erythromycin (ERY), Imipenem (IMP), Ampicillin (ACX) Cefixime (ZEM) and Levofloxacin (LBC), Sensitive(S), Resistance(R), Intermediate(I)

Table 4: Antagonistic effects of isolates from abattoir against *Staphylococcus aureus* and *Escherichia coli*

S/No.	Antagonist	Pathogens	Result
1	*Klebsiella* species	*Staphylococcus aureus*	No antagonism
2	*Shigella* species	*Staphylococcus aureus*	No antagonism
3	*Bacillus* species	*Staphylococcus aureus*	No antagonism
4	*Proteus* species	*Staphylococcus aureus*	No antagonism
5	*Escherichia coli*	*Staphylococcus aureus*	No antagonism
6	*Klebsiella* species	*Escherichia coli*	No antagonism
7	*Shigella* species	*Escherichia coli*	No antagonism
8	*Bacillus* species	*Escherichia coli*	No antagonism
9	*Proteus* species	*Escherichia coli*	No antagonism

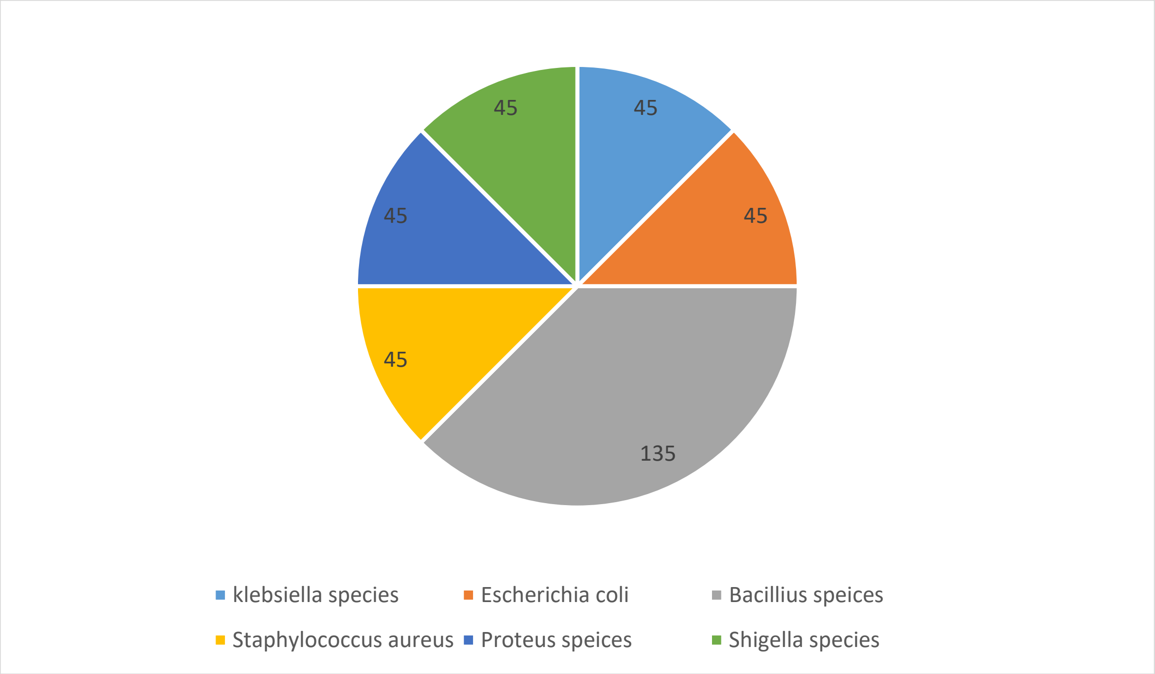

Fig 1: Pie chart showing the frequency of bacterial isolates from abattoir samples

DISCUSSION

Literature has shown that wastewater from abattoir can serve as a reservoir for resistant bacterial pathogens spread within the community if not well treated or disposed of (Imarenezor *et al*., 2017).

The high bacterial counts observed in this study shows presence of these organisms in the abattoir environment, or their facilities could be attributed to the fact that meat contains abundant nutrient requirements for the growth of microorganisms (Srikanth *et al.,* 2008).

The morphological and biochemical tests revealed biodiversity of both Gram positive and Gram-negative bacteria with some pathogenic and others non-pathogenic. The bacteria presumptively identified in this study were *Escherichia coli*, *Klebsiella* specie, *Shigella* species, *Bacillus* species, *Staphylococcus aureus,* and *proteus* species. These are similar to those reported by Omoya et al., 2014 who isolated S. aureus, B. subtilis, Micrococcus Sp. and E. coli from aerosols of an abattoir in Akure, Nigeria. E. coli is an enteric bacterium that is accountable for the epidemic of diarrhea. Presence of E. coli anywhere is usually an indication of poor hygiene. (Willey *et al.,* 2008). S. aureus can also result to a wide variety of diseases in man and animals by the synthesis of toxin. Staphylococcal toxins are known cause of food poisoning; this is because the toxins are produced by bacteria commonly found in inappropriately kept food items. (Willey et al.,2008). B. subtilis has been reported to cause disease in sternly immune compromised patients. It causes food poisoning and chronic skin infection, the presence of these bacteria in an abattoir environment depicts the poor state of hygiene and sanitary practices employed during the different stages of abattoir operations (Adekolurejo *et al*.,2016). The possible sources of contaminants could be due to the unhygienic manner of handling meat in abattoirs, and the badly managed environments where animals are daily slaughtered and processed. The conventional antibiotics tested in this study were those frequently administered for animal production. In this study, ciprofloxacin had the highest inhibitory effect on both Gram positive and Gram-negative bacteria, and thus could be recommended to infected workers that are exposed to these bacterial isolates.

In this study, we addressed the role of growth parameters on the cumulative production of BLIS by bacteria isolated from abattoir.

CONCLUSION

The data represented in the study demonstrated that there is not antagonism between the antagonist and the pathogen. Bacteriocin production is a defense strategy or adaptive mechanism that contribute to the success of niche colonization. In this study, all of the isolate tested were BLIS-, showing absence of inhibition patterns in relation to the species of producer isolates. Based on the findings of the study, the following recommendations are made; proper treatment of sludge wastewater should be done before disposal and channels should always be constructed for the disposal of sewage to avoid environmental pollution; this will help to maintain environmental sanity. Based on the find and conclusion it is therefore recommended thus;

1. Purification and Characterization: Isolate and characterize the bioactive compounds responsible for the antagonistic effects to understand their mechanisms of action.

2. In Vivo Testing: Evaluate the efficacy and safety of these compounds in animal models to assess their therapeutic potential.

3. Genomic Analysis: Conduct genomic studies to identify genes involved in the production of antimicrobial compounds and explore genetic engineering possibilities.

4. Application Development: Explore the potential application of these bacteria or their metabolites in bioremediation, agriculture, or pharmaceutical industries.

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Conflicts of interest

There are no conflicts of interest.

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