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Antibiogram of *Staphylococcus* Species Isolated from Some Abattoirs in Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Foodborne contamination especially by *Staphylococcus* species is of concern as many isolates of the genus have been reported for their multidrugs resistance. The study was aimed at determining the antimicrobial resistance of *Staphylococcus* species isolated from abattoir. A total of 100 samples were collected from five different surfaces; bucket swab, butchers hand swab, table swab, knife swab and meat swab from four abattoirs in of Rivers State, Nigeria. The samples were transported aseptically to laboratory for immediate analysis. The samples were cultured on mannitol agar (MSA) using standard microbiology technique to isolate *Staphylococcus* species. Hemolysis test was carried out and susceptibilities of isolates against a panel of 10 antibiotics were determined using the Kirby-Bauer disk diffusion method, and the multiple antibiotic resistance (MAR) index of the isolates were determined. Out of the 43 isolates of *Staphylococcus* from the abattoirs, 51.1% were coagulase positive while 48.9% were coagulase negative. 18.6% of the

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Staphylococcus species produced beta hemolysis and 16.28% showed alpha hemolytic activity. *Staphylococcus* species produced resistance to amoxicillin (88.4%), zinnacef (88.4%), ampiclox (88.4%), receptrin (60.5%), streptomycin (37.2%), septrin (37.2%), erythromycin (34.5%), and gentamicin (39.5%), ciprofloxacin (20.9%) and perfloxacin (16.8%). The multidrug resistance index (MAR index) showed that 2.35% of the isolates showed complete resistance to all the 10 antibiotics tested with MAR index of 1, 4.6% recorded MAR index of 0.1, 4.6% recorded MAR index of 0.2. Majority of the isolates of *Staphylococcus* species associated with meat and materials or equipment (such as bucket, knife) used in abattoir and the hands of butcher are multidrug resistance and is of great medical concern hence there is need to regulate the indiscriminate use of antibiotics.

Keywords: Staphylococcus species; abattoir; coagulase positive; antibiotics.

1. INTRODUCTION

"Foodborne infection is one of the leading causes of deaths with severe public health, economic, and social consequence worldwide" [1]. "The global foodborne diseases (FBDs) estimations conducted by the World Health Organization (WHO) indicate that about 30% of the populations in the industrialized countries suffer from food borned disease. Studies have reported that Africa has the highest burden of FBDs per population with 91 million related diseases and 137,000 death per annum and this has been worsened by antibiotic resistance associated with microorganisms that cause these foodborne diseases" [2]. "Interaction of food handlers with food-producing animals in the abattoir can also be a risk factor for the contamination of meat with zoonotic pathogens and further transmission within the food processing environment" (Normanno et al., 2015;) [3].

"Studies have reported that prolonged use and misuse of antimicrobial agents in agriculture, stock farming and in treatment of human diseases have resulted in rapid resistance of many bacteria to several antibiotics of different classes" [4].

"Staphylococcus species due on skin, glands and mucous membranes of almost all the warm blooded animals which prefer aerobic environment however can also grow in the absence of oxygen at temperature range of 6-44 °C (optimum 37 °C) and the range of pH is 4.2-9.3 (optimum 7)" [5]. "*Staphylococcus* species is an important opportunistic pathogen of humans and animals, and a leading cause of foodborne disease globally [1]. Food poisoning by the staphylococcal toxin is characterised by diarrhoea, nausea, abdominal cramping, and vomiting within 24 h of ingestion" [6].

"The tendency of microorganisms to persist and grow in the presence of antimicrobial substance at a concentration would inhibit their growth or kill them is referred to as, antimicrobial resistance. Of recent, antimicrobial resistance (AMR) has become a big threat to health globally. There is an increase level of antibiotic reported with the globe making it difficult to treat infectious diseases, prolong stay in the hospital and result in increase in cost of medical treatment" [7].

"The genes that encode resistance toward cephalosporins (blaCTX-M), penicillins (mecA and blaZ), glycopeptides (vanA and vanB), aminoglycosides (aacA-D), macrolides (ermA, ermB, msrA, and msrB), tetracyclines (tetK and tetM), folate pathway antagonists (dfrA1), ansamycins (rpoB), lincosamides (linA), fluoroquinolones (gyrA and grlA), phenicols (fexA) and streptogramins (vatA and vatB) have been reported in the *Staphylococcus* bacteria recovered from clinical and food samples. Antibiotic resistant-S. aureus strains caused more severe clinical diseases with higher morbidity and mortality rates for a longer period, which accommodate higher economic loads of control, prevention, and treatments" [8].

"The spread of resistance to antimicrobial agents in *Staphylococcus aureus* is largely result from the acquisition of plasmids and or transposons. Plasmid which is extrachromosomal genetic particle allows the movement of genetic material including antimicrobial resistance genes between bacterial species and genera" [9]. This study was aimed at understanding the prevalence of antibiotic resistance of *Staphylococcus* species from some abattoirs.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The sampling was conducted in four abattoirs; two from Obio/Akpor and two from Eleme Local Government of Rivers State and written

authorization from the butchers was obtained. Samples were obtained from five different surfaces: Bucket swab, butchers hand swab, table swab, knife swab, meat swab. All experiment was performed in duplicate under aseptic conditions. The sterile swabs were soaked in peptone solution, and rubbed on the surface. After the friction swabs were placed individually into previously identified tubes containing 10ml of sterile peptone solution, and put in icebox cooler and taken to the laboratory for further study (Lara *et al.,* 2019).

2.1.1 Culturing and isolation of *Staphylococcus* spp.

Serial dilution was carried out by dispensing 9ml of already prepared normal saline into three test tubes labeled 10^{-1} – 10^{-3} and autoclaved at 121^{o}C for 15 minutes at a pressure of 15 pounds per square inch (psi). After cooling, the samples were inoculated into the first test tube labeled 10^{-1} and properly mixed. A sterile pipette was used to pipette 1ml of mixture was taken from the 10^{-1} dilution and dispensed into the second tube to dilution of 10^{-2}. The same process was then repeated for the remaining tube, taking 1ml from the previous tube and adding it to the next 9 ml diluents. An aliquot (0.1mL) from the appropriate dilution was inoculated into pre-dried MSA plates using spread plate method. The inoculated plates were incubated at 37°C for 24 hrs. After incubation, yellow colonies were counted and recorded as *Staphylococcus* counts (APHA, 2012).

2.1.2 Characterization and identification of isolated bacteria

After 24 hour incubation, bacteria isolates were characterized based on colonial appearance on Manitol salt agar. Gram stain was done to determine Gram reaction and bacterial morphology. After gram staining those colony shown to be gram positive cocci were subjected to further biochemical tests. Overnight culture of the isolates was used for biochemical test. Biochemical tests carried out were catalase and coagulase tests.

2.2 Determination of Antimicrobial Susceptibility Profile

The antimicrobial susceptibility of *Staphylococcus species* to antibiotic discs (10 µg ciprofloxacin, 15 µg erythromycin, 10 µg gentamicin, 10 µg receptrin, 30 µg amoxicillin, 30µg ampiclox and 30µg septrin, 20µg, Streptomycin, 25µg Zinnacef and 25µg perfloxacin) was determined using the Kirby-Baur disc diffusion method and interpreted according to the 2020 Clinical and Laboratory Standards Institute (CLSI) guidelines. One to two pure colonies of the organism grown overnight on nutrient agar were suspended into 2mLs of physiological normal saline to make a 0.5 McFarland Standard. These bacteria were then spread evenly on a Mueller Hinton agar plate using a sterile swab. After allowing the plate to air dry for a few minutes, antimicrobial discs were gently placed on the Mueller Hinton agar. The plates were then incubated for 16 to 24 h at 37^{o}C. The result was read by measuring the diameter of the zone of inhibition.

2.2.1 Risk assessment parameters of *Staphylococcus* species

The Multiple Antibiotic Resistance (MAR) index was calculated as (a/b) where ‘‘a’’ is the number of antibiotics in which the isolates were resistant to, and ‘‘b’’ is the total number of antibiotics to which the isolate was exposed.

2.3 Test for Haemolysis

Blood agar was used in the hemolysis test adopting the method of Amini et al. [10]. Pure culture of the isolates of Staphylococcus was streaked on the freshly prepared Blood agar. The cultured media were incubated at 37°C for 24h. Then, the haemolysis production was observed in diameter around the colonies on plates.

2.4 Data Analysis and Interpretation

The data obtained from the study were analyzed using descriptive statistics and chi square. A statistical package INSTAT 3a was used for the analysis.

3. RESULTS

The prevalence of species of *Staphylococus* species between the sampled materials used in different abattoirs is shown in Fig. 1. The highest prevalence (46%) was recorded in the bucket and the least prevalence (7%) was recorded in the swabbed hands of the butcher. Coagulase positive and coagulase negative *Staphylococcus* species were identified and the result is shown in Table 1. Out of the 43 isolates of *Staphylococcus* from the abattoirs in the course of the study, 22(51.1%) were coagulase positive while 21 (48.9%) were coagulase negative. The hemolysis

of the isolates from the samples is shown in Table 2. Most (65.12%) of the isolates did not produce visible hemolysis (gamma hemolysis) while 18.6% produced beta hemolysis and 16.28% showed alpha hemolytic activity. The antibiotic susceptibility of the staphylococcal isolate as represented in Table 3 shows that the *Staphylococcus* species produced high resistance to amoxicillin (88.4%), zinnacef (88.4%), ampiclox (88.4%), receptrin (60.5%), streptomycin (37.2%), septrin (37.2%), erythromycin (34.5%), and gentamicin (39.5%). The isolates exhibited low resistance to ciprofloxacin (20.9%) and perfloxacin (16.8%). The multidrug resistance index (MAR index) of the *Staphylococcus* species is shown in Table 4. 2.35% the isolates showed complete resistance to all the 10 antibiotic tested. Out of the isolates tested against the antibiotics, 4.6% recorded MAR index of 0.1 while another 4.6% recorded MAR index of 0.2 hence, 9.2% of the isolate were not multidrugs resistance to the antibiotics test whereas, 90.8% of the isolates produced multidrug resistance to the antibiotic tested as they recorded MAR index above 0.2. Bacteria having MAR index >0.2 originate from a high-risk source of contamination where several antibiotics or growth promoters are used, while values <0.2 show bacteria from source with less antibiotic use. A completely resistant isolate has an MAR index of 1.0.

Table 1. Distribution of coagulase among the abattoirs

Abattoir	Coagulase positive	Coagulase negative	Total
Akpajor	5	6	11
Aleto	7	3	10
Rumuokoro	3	8	11
Rumuokwurushi	7	4	11
Total	22	21	43

Table 2. Percentages of haemolysis among the isolates from the different abattoir

Abattoir	Haemolysis			
	Alpha (α)	Beta (β)	Gamma(γ)	Total
Akpajor	1(2.33%)	2(4.65%)	8(18.61%)	11(25.58%)
Aleto	1(2.33%)	1(2.33%)	8(18.61%)	10(23.26%)
Rumuokoro	1(2.33%)	3(6.98%)	7(16.28%)	11(25.58%)
Rumuokwurushi	4(9.30%)	2(4.65%)	5(11.63%)	11(25.58%)
Total	7(16.28%)	8(18.61%)	28(65.12%)	43(100%)

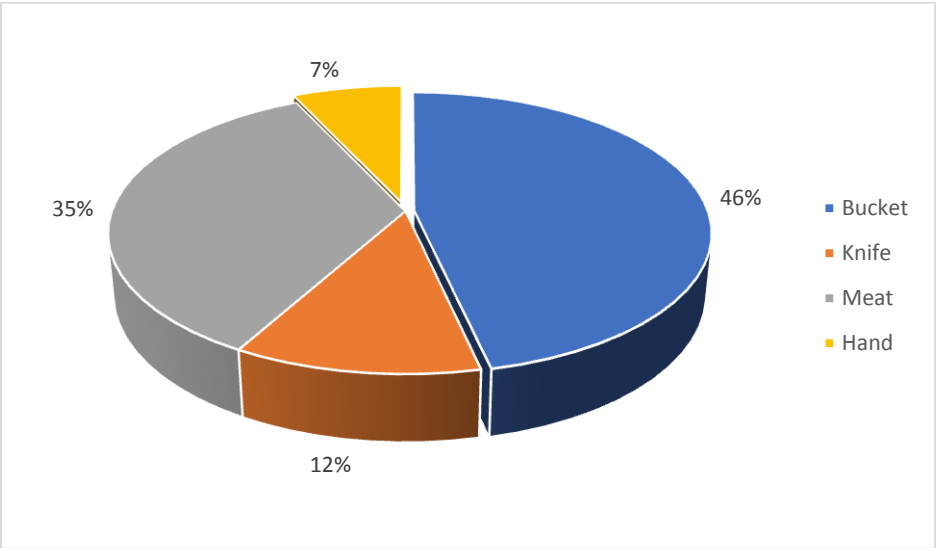

Fig. 1. Prevalence of Staphylococcus species among the different samples in the abattoir

Table 3. Antibiotic resistance pattern of *Staphylococcus* species (n=43) isolated from the different abattoir studied

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
AM	38(88.4)	1(2.3)	4(9.3)
R	26(60.5)	5(11.6)	12(29.9)
APX	38(88.4)	3(7.0)	2(4.7)
CPX	9(20.9)	0(0.00)	34(79.1)
S	14(32.6)	6(14.0)	23(53.5)
SXT	16(37.2)	1(2.3)	26(60.5)
E	15(34.9)	4(9.3)	24(55.8)
Z	38(88.4)	0(0.00)	5(11.6)
PEF	8(16.8)	1(2.3)	34(79.1)
CN	17(39.5)	4(9.3)	22(51.2)

KEY: AM- Amoxicillin, R- Rocephin, APX- Ampiclox, CPX- Ciprofloxacin, S- Streptomycin, SXT- Septrin, E- Erythromycin, Z- Zinnacef, PEF-Perfloxacin, CN-Gentamicin

Table 4. Multiple Antibiotic Resistance Index (MAR Index) of *Staphylococcus* species

MAR Index	Number of the isolates
0.1	2 (4.6%)
0.2	2 (4.6%)
0.3	8 (18.6%)
0.4	9 (20.9%)
0.5	5 (11.6%)
0.6	4 (9.3%)
0.7	5 (11.6)
0.8	4 (9.3%)
0.9	3 (6.97%)
1.0	1 (2.35)

4. DISCUSSION

In this study, *Staphylococcus* species was ubiquitously present in the different samples including, bucket, knife, hand of the butcher as well as the beef (meat). These results suggest that *Staphylococcus* species is highly resilient and adaptive to grow in different conditions [11]. From the result of the prevalence of the different species of *Staphylococcus* species between the materials sampled, bucket samples had the highest prevalence and these can be related to the use of this materials in the abattoir by different workers and unhygienic condition of the materials. The result recorded in this study is contrary to the report of Adugna et al. [12] which reported that knife had the highest prevalence of 22.5% compared to others materials used in the abattoir. The difference can be relative of the frequency in the use of these materials and the unhygienic conditions of among abattoirs (Tsepo *et al*., 2016)

The *Staphylococcus* genus includes a heterogeneous group of Gram-positive bacteria. The genus comprises 81 species and subspecies divided in the two groups, coagulase-positive and coagulase-negative, based mainly on clinical and diagnostic aspects (Haag *et al.,* 2017). Coagulase-positive *Staphylococcus* are well recognized as important human and animal pathogens, while the role of coagulase-negative *Staphylococcus* species as primary pathogens or opportunistic bacteria are subject of many studies [13]. Coagulase positive *Staphylococcus* species are identified as *Staphylococcus aureus* while other species of *Staphylococcus* are categorized as coagulase negative *Staphylococcus* [14]. The result obtained in this study showed that 51% of the isolates where coagulase positive compared to 48.9% recorded for coagulase positive. This is in contrast to the result recorded in the study of Virdis et al. [14] in which majority of the isolates (82.6%) were coagulase negative isolated from goat.

The virulence by some bacteria is excited by and some enzymes that aid in their invasion into the host cells. Hemolysin is an important virulence factor produced by many bacterial species [15]. The hemolysins and leukocidins released by *Staphylococcus aureus* are of the famous systolic toxins. The bacterium produces different hemolytic toxins but different isolates produce different amounts of these toxins [10]. *Staphylococcus* hemolysins are important virulence factors, with cytotoxic activity, that contribute to cell membrane damage and the lysis of keratinocytes. α-hemolysin is an important pore-forming cytolysin with a high-affinity for mammalian cells, while β-hemolysin is a sphingomyelinase that hydrolyzes plasma membrane lipids and has a lytic activity that is not as efficient as that of other hemolysins [13]. In this study, 18.6% of the isolates were able to

exhibit β-hemolysis in comparison to 16.2% which produced α-hemolytic activity while 65.12% of the population showed no hemolysis (γ-hemolysis) however, both β-hemolysis and α-hemolysis contribute to Staphylococcal pathogenicity and are linked with virulence hence on the basis of hemolysis, it can be reported that 34.8% of the isolates obtained in this study had the virulence factor, hemolysin. Alpha toxin is also considered as a main factor for developing beta hemolysis area in blood agar [10] and according to the result obtained in this study, 16.2% of the *Staphylococcus* species can be linked with the ability to produce alpha toxin. In the study of Amini et al. [10], the frequency to produce alpha toxin was reported to be 85% by *Staphylococcus* species.

Antibiotic susceptibility testing showed various levels and cross-resistance to commonly used antibiotics. From the study, there was a higher level of resistance to amoxicillin (88.4%), ampiclox (88.4%) and receptrin (60.5%). This is similar to the study of Deyno *et al*. (2017) which reported that 90.9% of *Staphylococcus* species were resistant to amoxicillin. The high resistance recorded by the microorganisms to these antibiotics can be attributed to the indiscriminate use of these antibiotics leading to the inducement of resistance. Ampiclox is one of the most abuse antibiotis in Nigeria and the percentage increase in resistance cannot be unrelated to their uncontrollable usage [4].

The lower resistance of the isolates of *Staphylococcus* species to ciprofloxacin antibiotics as recorded in this study is in line with the study of Thwala et al. [6] in which ciprofloxacin was observed to have shown less resistance to by *Staphylococcus* species isolated from meat and meat products. Most of the isolates in this study showed high sensitivity to antibiotics, perfloxacin, ciprofloxacin and Septrin. This implies that such antibiotics can be used to treat infections caused by *Staphylococcus* sp. This is also line with the study of Gizaw et al. [11] which report the majority (96.4%) of *Staphylococcus* species were susceptible to ciprofloxacin. Kandel et al. [16] all reported that 64.1% of *Staphylococcus* species were susceptible to ciprofloxacin. In other studies, ciprofloxacin being a broad-spectrum antibiotic had been reported to be more sensitive to species of *Staphylococcus* [4]. Perfloxacin is flouroquinolone base antibiotic and have been reported to be effective against some antibiotic resistant bacteria. Chikwendu et al. [17] in a study reported a less (0.05%) resistance rate of bacteria to perfloxacin.

The MAR index is an important risk assessment tool in determining the susceptibility ratio of microorganism to drugs, and the value of the MAR index (nominally 0.2) has been applied to differentiate low- and high-risk regions where antibiotics or growth promoters are overused [18]. The analysis of MAR index indicates the number of bacteria showing antibiotic resistance in the risk zone of the susceptibility study [1]. The majority (90.8%) of the isolates in this study exhibited a multidrug resistance phynotype to amoxicillin (88.4%), Ampiclox (88.4%), Receptrin (60.5%). Asante et al. [19] in their study reported that 76.4% of *Staphylococcus* isolate were multidrug resistance to common antibiotics used. Highest MAR index was observed in 2.35% of the isolates where an MAR index of 1 was recorded. This suggest that the isolates were produced resistance to all the antibiotics tested. This could be attributed to possession of multiple resistance genes in the bacterial genome that enable them resist all the antibiotics [20]. This is in line with the findings of Ismail et al. [9] who reported that MAR by *S. aureus* is usually associated with increased expression of multiple antibiotic resistance genes. Multidrug resistance in one of the most prevalence bacteria, *Staphylococcus* species from food or clinical source is of much concern as highlighted in this study.

5. CONCLUSION

According the result obtained in this study, majority of the isolates of *Staphylococcus* species associated with meat and materials or equipment (such as bucket, knife) used in abattoir and the hands of butcher are multidrug resistance and is of great medical concern however some antibiotic such as ciprofloxacin and perfloxacin proved effective to majority of the species. Efficient regulation of antibiotics in food animals which will result in decreased resistance in bacteria from food source should be implemented.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Amoako DG, Somboro AM, Abia ALK, Molechan C, Perrett K, Bester LA, Essack

SY. Antibiotic resistance in *Staphylococcus aureus* from poultry and poultry product in uMgumgundlovu district South African using farm to fork approach. Microbial Drugs Resistance. 2019;2:1-11.

2. World Health Organisation. WHO's first ever global estimates of foodborne diseases find children under 5 account for almost one third of deaths. Geneva, Switzerland; 2015.
Available:www.who.int/news-room/detail/03-12- 2015/
Access on February 9, 20
3. Odetokun IA, Ballhousen B, Adetanji VO, Ghali-Mohammed I, Adelowo TM, Adetanji SA, Fetsch A. *Staphylococcus aureus* in two municipal Abattoir in Nigeria. Risk Perception, Spread and Public Health Impications. Veterinary Microbiology. 2018;216:52-59.
4. Pekana A, Green E. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from meat carcases and bovine milk in Abattoir and diary farm of Eastern Cape South Africa. International Journal of Environmental Research and Public Health. 2018;15:2223-2246.
5. Deyno S, Toma A, Worku M, Bekele M. Antimicrobial resostamce profile of *Staphylococcus aureus* isolates from ear discharges of patients at University of Hawassa Comprehensive Specialized Hospital. BMC Pharmacology, and Toxicity. 2017;18:35-42.
6. Thawla T, Madorota E, Basson A, Butaye P. Prevalence and characteristic of *Staphylococcus aureus* associated with meat and meat products in African Countries: A review. Antibiotics, 2021;10:108-124.
7. Afunwa RA, Ezeanyinka J, Afunwa EC, Udeh AS, Oli NA, Unachukwu M. Multiple antibiotic resistant index of gram-negative bacteria from bird droppings in two commercial poultries in Enugu, Nigeria. Open Journal of Medical Microbiology. 2020;11:171-181.
8. Mashak Z, Tavakoli-Far B. Antimicrobial resistance properties of *Staphylococcus aureus* isolates from powdered packaged medical plants and bottle herbal distillate. Research Square. 2020;3:1-17.
9. Ismail HY, Bello HS, Mustafa A, Adamu A. Multidrug Resistance Pattern of *Staphylococcus aureus* isolates in Maidugiri Metropolis. Scientific Review. 2015;1(2):16-20.
10. Amini A, Asghar AA, Ghaemi EA, Rahmi S, Koohsar F, Kazemi R, Koahasari E, Roastoei M. Comparison of ferment sugars, producing haemolysis and measuring growth in methilin-resistant and methicillin-sensitive *Staphylococcus aureus* isolates from inpatients and healthcare workers in Gorgan Hospitals, North Iran. Bioscience Biotechnology Research Asia. 2013;10(1):77-84.
11. Gizaw F, Kekeba T, Teshome F, Kebede M, Abreham T, Hayishe H, Waktole H, Tufa Mammo, Edao BM, Ayana D, Abunna F, Beyi FA, Abdi RD. Distribution and antimicrobial resistance profile of coagulase-negative *Staphylococcus* from Cattle, Equipment and Personnel on Dairy Farm and Abattoir Setting. Heliyon. 2020;6:1-11.
12. Adugna F, Pal M, Girmay G. Prevalence and antibiogram assessment of *Staphylococcus aureus* in beef at municipal abattoir and butcher shop in Addis Ababa, Ethiopia. Biomed Research International; 2018.
13. Bertelloni F, Cagnoli G, Ebani VV. Virulence and antimicrobial resistance in Canine *Staphylococcus spp* isolates. Microorganism. 2021;9:515-526.
14. Virdis S, Scarano C, Cossu F, Spanu V, Spanu C, De Santos EPL. Antibiotic resistance in *Staphylococcus* and coagulase negative *Staphylococcus* isolate from goat with subclinical masulis. Veterinary Medicine International. 2010;17:1-6.
15. Mongrovejo DC, Perini L, Gostincar C, Sepcic K, Turk M, Ambrozic-Avgustin J, Brill FAH, Gunde-Cimerman N. Prevalence of antimicrobial resistance and hemolytic phenotypes in culturable artic bacteria. Frontier in Microbiology. 2020;11:570-553.
16. Kandel SN, Adhikari N, Dhungel B, Shrestha UT, Angbuhang KR, Karki G, Adhakari B, Banjara, MR, Rijal KR, Ghimire P. Characteristics of *Staphylococcus aureus* isolated from clinical specimens in tertiary hospital, Kathmandu, Nepal. Microbiology insights. 2020;13:1-6.
17. Chikwendu CI, Nwabueze RN, Anyanwau BN. Antibiotic resistance profile of *Escherichia coli* from clinically healthy pig and their commercial farm environment. African Journal of Virology Research. 2008;2(2):142-147.

18. Riaz S, Faisal M, Hasnain S. Antibiotic susceptibility pattern and Multiple Antibiotic Resistances (MAR) calculation of extended spectrum-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella species* in Pakistan. African Journal of Biotechnology. 2011;10:6325–6331.
19. Asante J, Hetsa BA, Amoako DG, Abia ALK, Bester LA, Essack SY. Multidrug-resistant coagulase-negative staphylococci isolated from bloodstream in the uMgungundlovu district of KwaZulu-Natal Province in South Africa: Emerging pathogens. Antibiotics. 2021;10:198-208.
20. Haag AF, Fitzgerald JR, Penadés JR. *Staphylococcus aureus* in animals. Microbiol. Spectr. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, 30th ed.; Supplement M1002020; CLSI: Tehran, Iran; 2019.

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