



ORIGINAL ARTICLE

OCCURRENCE OF MULTIDRUG RESISTANCE IN COAGULASE POSITIVE METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM OPEN WOUNDS IN DOGS IN JOS, NORTH CENTRAL NIGERIA

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published in July 14, 2020 (<https://doi.org/10.1371/journal.pbio.3000410>), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Staphylococcus aureus is an important cause of wound infections in companion animals, and infections with methicillin-resistant *Staphylococcus aureus* (MRSA) are of particular concern due to limited treatment options and their zoonotic potentials. The aim of this study was to investigate the occurrence of methicillin-resistant *Staphylococcus aureus* in open wounds in dogs brought for routine veterinary care in Jos and environs. Only coagulase positive *Staphylococcus aureus* isolates were used to determine the antimicrobial susceptibility using 8 antibiotics. These were found to be resistant to 4 antimicrobials; penicillin, methicillin, ceftiofur and oxacillin, at the concentrations tested. Suggesting that, the *Staphylococcus aureus* were methicillin resistant phenotypes. However, the isolates also showed sensitive and intermediate susceptibility to gentamicin, with only one coagulase positive *Staphylococcus aureus* isolate showing resistance to gentamicin. Similarly, the results on kanamycin susceptibility indicated majority of the isolates were susceptible, with only one isolate showing intermediate susceptibility, however few of the isolates were resistant. All the isolates showed resistance to cephalothin. Similarly, resistance to neomycin was observed in almost all the isolates, except two isolates, that were found to be sensitive. Sensitivity to ceftiofur was observed in 10 out of the 13 isolates; remaining 3 isolates were resistant to ceftiofur. The results showed that methicillin-resistant *Staphylococcus aureus* occurred in open wounds in dogs and could be a recurring issue in clinical management of wound infections in dogs, and a risk to public health.

Key words: coagulase positive; dogs; methicillin resistance; open wound; *Staphylococcus aureus*

INTRODUCTION

Staphylococci are Gram-positive bacteria, and they are classified into two groups, coagulase-positive (CoPS) and coagulase-negative (CoNS), based on their ability to produce the enzyme coagulase [1]. *Staphylococcus aureus* and *Staphylococcus pseudintermedius* are the most important species in the CoPS group as they are major pathogens for both humans and animals, especially *S. aureus*. Although CoNS are saprophytic and rarely pathogenic [5], multidrug-resistant (MDR) strains have been associated with severe cases of difficult to treat infections, especially in immunocompromised individuals [19].

Methicillin-resistant staphylococci (MRS) are among the most important bacteria in both human and veterinary medicine and of major clinical, public health and economic concern [7]. The problem is aggravated by the fact that MRS, in addition to β -lactam antibiotics, are commonly resistant to other classes of antimicrobial agents, including aminoglycosides, macrolides, phenicols, tetracyclines and fluoroquinolones [8,6]. Methicillin resistance is conferred by the *mecA* gene which encodes an altered penicillin-binding protein (PBP2a or PBP2') with a low affinity for β -lactam antimicrobials [18].

Even though Chah et al., 2014 [3] have characterized methicillin-resistant CoNS from dogs in Nsukka, Nigeria, there are, however, no documented reports on the characterization of CoPS methicillin-resistant staphylococci from dogs in Nigeria. Thus, we conducted a small survey to investigate the occurrence of CoPS methicillin-resistant *Staphylococcus aureus* from clinical wound infections in dogs in Jos, North Central Nigeria. The MRSA was identified from phenotypic *Staphylococcus aureus*-positive samples subjected to antimicrobial susceptibility testing to determine their resistance to methicillin. Due to the fact, that we could not find a literature reporting CoP MRSA in open wounds in dogs in Nigeria, this represents the first report on CoP MRSA in dogs in Jos, North Central Nigeria. The data provides important baseline measurements for future surveillance of CoP MRSA in dogs in Nigeria. The multidrug resistance pattern observed in this study is important in guiding clinical judgement on which antibiotic to use for the treatments of wound infections in dogs in the study area.

MATERIALS AND METHODS

Study area

Dogs from Jos and Bukuru were included in the study. A total of 100 dogs with open wounds presented to the ECWA Veterinary clinic, Bukuru Jos Metropolis for routine veterinary care were sampled.

Sample collection, preservation and transportation

The cotton tip of each sterile swab stick was moistened in sterile normal saline and gently rolled over the wound for about 10 seconds and transferred into bijoux bottle containing transport media; the samples were adequately labelled. All samples were transported on ice packs to the Bacteriology Division, National Veterinary Research Institute, Vom for processing.

Bacterial culture, isolation, and identification

Each swab sample was inoculated and streaked on mannitol salt agar (Oxoid, Basingstoke, UK). All inoculated plates were incubated at 37 °C from 24 to 48 h. On each plate that produced growth, discrete colonies were randomly selected and purified on nutrient agar.

Purified colonies were subjected to Gram's staining and catalase test and presumptive staphylococcal colonies were further tested for coagulase production (slide coagulase test using rabbit plasma), DNase activity, and hemolysis on 5 % sheep blood agar using standard procedures [12]. Representative colony which appeared yellow on mannitol salt agar and were gram positive cocci was subjected to catalase test for preliminary confirmation of *Staphylococcus* species. Those colonies positive for catalase test were further tested for coagulase activity to confirm *Staphylococcus aureus* from other nonpathogenic *Staphylococci*. Isolates were preserved on nutrient agar slants for further analysis, including antimicrobial susceptibility test.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing of *Staphylococcus aureus* was performed using disc diffusion method as described [2]. Antibiotic discs used included: penicillin, methicillin, oxacillin, gentamicin, kanamycin, neomycin, cephalothin and ceftiofur in various disc concentrations. The minimum inhibitory concentration results were interpreted based on the approved standards of the Clinical

and Laboratory Standards Institute [16,17]. All MRSA positive isolates were stored in glycerol stocks at -80°C at Bacteriology Division National Veterinary Research Institute, Vom.

Statistical analysis

Data obtained were imputed into Microsoft excel 2010 and percentage of resistance showed by the isolates to various antibiotics used were calculated.

RESULTS

The outcome of the study is highlighted below.

Demography of dogs

Out of the 100 dogs sampled, 62 and 33 were male and female dogs, respectively. Sampled dogs were 62 and 23 adult and young dogs, respectively. The breed demography indicated that 54 were foreign breeds and 31 were local breeds. This shows that high numbers of dogs brought for veterinary care in the study area during the period of the study were foreign breeds compared to local breeds that were in low numbers (Fig. 1).

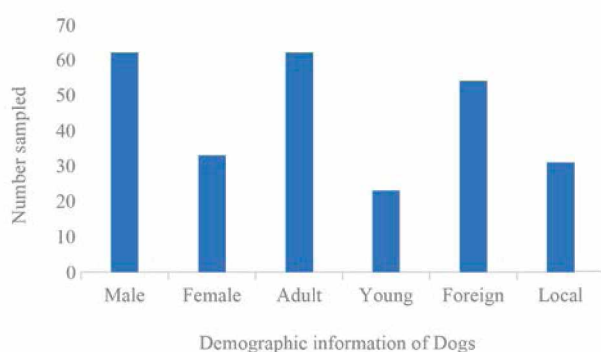


Fig. 1. Demography of the dog samples based on breed, sex and age.

Cultural characteristics of *Staphylococcus aureus* isolated from open wounds in dogs

All samples (N = 100) processed grew on mannitol salt agar producing yellow colonies suggestive of *Staphylococcus* species; of these, 13 were coagulase positive, and 17 showed beta hemolytic activity. However, none of the isolates were positive for DNase activity. Only 13 out of the 17 beta hemolytic staphylococci isolated were coagulase positive. Thus, only 13 beta hemolytic coagulase pos-

itive staphylococci were used for the antibacterial susceptibility. These two characteristics indicated that the isolates were pathogenic. Therefore, determining their sensitivity to antibiotics is crucial.

Antimicrobial susceptibility of *Staphylococcus aureus* isolated from open wounds in dogs

We have performed disc diffusion test on all coagulase positive *Staphylococcus aureus* isolates to determine their susceptibility to 8 antibiotics (Table 1). Resistance to 3 antibiotics (penicillin, methicillin and oxacillin) was observed in all the *Staphylococcus aureus* isolates at the concentrations tested (Table 1). The result showed *Staphylococcus aureus* isolates were resistant to methicillin at the tested concentrations. However, 6 and 4 of the *Staphylococcus aureus* isolates tested for susceptibility to gentamicin were found to be sensitive and showing intermediate sensitivity to gentamicin, respectively. Perhaps only one *Staphylococcus aureus* isolate was resistant to gentamicin at the concentration tested (Table 1). Similarly, the results on kanamycin susceptibility showed that majority of the isolates were susceptible, with only one isolate showing intermediate susceptibility, however few of the isolates were resistant. All the isolates showed resistance to cephalothin and neomycin. However, two isolates showed sensitivity to neomycin. 10 out of the 13 isolates showed sensitivity to ceftiofur of which the remaining 3 isolates were resistant. In all, the susceptibility pattern showed that at least 63.3 % and 36.36 % of the isolates were susceptible to at least two (2) and one (1) antimicrobials, respectively. Similarly, 62 %, 23 %, 8 % of the isolates tested showed resistance to 3, 4, 5, and 2 antimicrobials, respectively.

The resistance profile showed in Table 2 indicates that all isolates showed complete resistance to penicillin, methicillin and oxacillin. However, nearly all the isolates were susceptible to kanamycin, with very few of the isolates showing resistance.

The isolates showed greater phenotypic intermediate resistance to kanamycin, and lower to-gentamicin. However, greater number of the isolates were sensitive to gentamicin.

The results in Table 4 indicated that 69.2 %, 23.07 % and 7.67 % of the coagulase positive MRSA showed phenotypic resistance to 3, 4 and 5 antibiotics, respectively. Overall, patterns of phenotypic resistance were summarized in Table 3.

Table 1. Antibiotic Susceptibility profile of coagulase positive MRSA isolated from open wounds in dogs in Jos, North Central Nigeria

Sample ID	Antibiotic susceptibility pattern based on RIS							
	Methicillin (30)	Oxacillin (1)	Penicillin (30)	Kanamycin (30)	Gentamicin (30)	Neomycin (30)	Cephalothin (30)	Ceftiofur (30)
EJS/002/2021	R	R	R	S	S	R	R	S
EJS/003/2021	R	R	R	R	S	I	R	S
EJS/007/2021	R	R	R	I	I	R	R	S
EJS/008 /2021	R	R	R	S	S	R	R	S
EJS/009 /2021	R	R	R	R	R	R	R	S
EJS/010/2021	R	R	R	S	S	I	R	S
EJS/011/2021	R	R	R	S	R	R	R	R
EJS/015/2021	R	R	R	S	S	R	R	S
EJS/019/2021	R	R	R	S	S	R	R	R
EJS/020/2021	R	R	R	S	I	R	R	S
EJS/021/2021	R	R	R	S	R	R	R	R
EJS/022/2021	R	R	R	S	I	R	8	S
EJS/027/2021	R	R	R	S	S	R	R	R

R= resistance, I = intermediate, S = sensitive

Table 2. Antibiotic susceptibility pattern based on resistance, intermediate and sensitive susceptibility (RIS)

Sample ID	Zone of inhibition (mm) of antibiotics (µg)							
	Methicillin (30)	Oxacillin (30)	Penicillin (30)	Kanamycin (30)	Gentamicin (30)	Neomycin (30)	Cephalothin (30)	Ceftiofur (30)
EJS/002/2021	6	6	9	24	18	13	11	34
EJS/003/2021	6	6	6	12	20	15	6	27
EJS/007/2021	6	6	8	16	14	9	9	30
EJS/008 /2021	6	6	10	23	19	12	12	40
EJS/009 /2021	6	6	10	10	12	12	6	28
EJS/010/2021	6	6	6	22	19	14	6	28
EJS/011/2021	6	6	6	18	12	10	6	6
EJS/015/2021	6	6	6	20	18	13	6	26
EJS/019/2021	6	6	6	18	16	12	6	8
EJS/020/2021	6	6	7	18	14	9	10	30
EJS/021/2021	6	6	6	19	0	10	6	16
EJS/022/2021	6	6	6	19	14	10	8	32
EJS/027/2021	6	6	6	18	15	13	6	17

DISCUSSION

Companion animals, particularly dogs and cats, are frequently implicated as potential reservoirs of methicillin-resistant staphylococci [15]. Studies on Staphylococcal species in companion animals is important because of their potential for zoonotic infections and possibility of resistance genes transfer [13].

Our study aimed at investigating the occurrence of MRSA in an open wound in dogs brought to the clinics by

families for routine veterinary care. One hundred samples were collected and processed.

In this study, the demographic characteristics of the dogs studied showed, out of the one hundred dogs sampled, sixty-two were male dogs and thirty-three were female dogs. Although our study was not a dog's population study, our results were not in concordance with the findings of Ogbu et al., [11] who reported female dogs were more prevalent than the male dogs (1:1.2) in the study area. This small survey which was mainly carried out to

Table 3. Intermediate and sensitive resistant phenotypes in coagulase positive MRSA isolated from open wounds in dogs in Jos, North Central Nigeria

Sample ID	Intermediate	Sensitive
EJS/002/2021	K	CN
EJS/003/2021		CN
EJS/007/2021	K	CN
EJS/008 /2021	K	CN
EJS/009 /2021		
EJS/010/2021	K	CN
EJS/011/2021	CN K	
EJS/015/2021	K	CN
EJS/019/2021	K	CN
EJS/020/2021	CN K	
EJS/021/2021	K	
EJS/022/2021	CN K	
EJS/027/2021	K	

CN = Gentamicin; K = Kanamycin

Table 4. Percentage of phenotypic antibiotic resistance of coagulase positive MRSA isolated from open wounds in dogs in Jos, North Central Nigeria

Sample ID	Resistant phenotypes	No. resistant	(%)
2	PEN MET OX	3	(69.23)
3	PEN MET OX K	4	
7	PEN MET OX	3	
8	PEN MET OX	3	
9	PEN MET OX K CN	5	(7.67)
10	PEN MET OX	3	
11	PEN MET OX	3	
15	PEN MET OX	3	
19	PEN MET OX	3	
20	PEN MET OX	3	
21	PEN MET OX CN	4	
22	PEN MET OX	3	
27	PEN MET OX CN	4	(23.07)

PEN = Penicillin; MET = Methicillin; OX = Oxacillin; K = Kanamycin; CN = Gentamicin

determine *Staphylococcus aureus* colonization of open wounds in dogs and the susceptibility or resistance of the isolates to methicillin and only used a small number of dogs (N = 100) brought for routine veterinary care in some selected veterinary clinics in the study area. Therefore, it may not present the exact population of dogs reported by Ogbu et al. [11]. Similarly, Obogbulam and Nwakonobi [10] and Rana et al. [14] have reported higher number of female dogs compared to male dogs in their studies of dogs' population in Lagos urban and rural areas. Their results are also not in accordance with our study. This showed preference for female over male dogs. This may be attributed to use of female dogs for breeding and cultural purposes [11].

The age characteristics of the dogs sampled in this study were sixty-two (62) for adult dogs and thirty-three (33) for young dogs. The report of the study conducted on dog population in the study area based on age showed majority of the dogs were adult (48.6 %), above 12 months of age. This was due to the fact that younger dogs were sold out after weaning, due to breeding value or security reasons. The same reason was observed by Rana et al. [14] who reported that most dog owners were mostly interested in security or companionship purposes.

Visits at the clinics for routine veterinary care were higher in exotic than local breeds. Out of the hundred dogs sampled, fifty-four (54) were foreign or exotic breeds and thirty-one (31) were local breeds. In their dog population

study, Ogbu et al. [11] had reported high population of indigenous breed (71.3 %) compared to local and mixed breeds (28.9 %) in the study area. They observed that indigenous breeds are kept mainly for cultural beliefs and delicacy. Exotic breeds are mainly kept by the elites who can afford their feeding, housing and veterinary care compared to local or mixed breeds whose owners are majorly poor and allow their dogs to freely roam and scavenge for their foods. They also observed that dogs are mainly kept for security reasons due to increased security challenges. Guard duties have been identified as the primary reason for keeping dogs in a number of states in Nigeria [10].

In our microbiological study for methicillin resistance, the samples collected were processed by culture, and identification of the isolates were performed using phenotypic characteristics on selective media, morphology, and biochemical tests such as catalase, coagulase and DNase tests. All the samples (N = 100) cultured grew luxuriantly on mannitol salt agar producing phenotypic yellow colonies, suggestive of *Staphylococcus* species. The result showed that 63 (63%) isolates were catalase positive and 13 (21 %) were coagulase positive, respectively, and none of the isolates were positive for DNase activity. Beta hemolytic activity on 5 % sheep blood agar was observed in seventeen (4) of the isolates recovered. The coagulase positive isolates were further identified as *Staphylococcus aureus*, based on their morphology and biochemical characteristics [11]. Coagulase positive *Staphylococcus aureus* also

frequently occurs in dogs [19]. Study has reported a prevalence of 16 % coagulase-positive *Staphylococcus aureus* in dogs in Bangladesh.

In our study, only the coagulase positive *Staphylococcus aureus* (COPS) was used to determine the antimicrobial susceptibility. The antibiotics susceptibility of the 13 coagulase-positive (CoPS) *Staphylococcus aureus* were determined using methicillin (30 µg), oxacillin (30 µg), cefoxitin (30 µg), penicillin (30 µg), gentamicin (30 µg), kanamycin (30 µg), neomycin (30 µg), cephalothin (30 µg), and ceftiofur (30 µg) discs. All the thirteen (13) coagulase-positive *Staphylococcus aureus* isolates tested showed resistance to penicillin, methicillin, cefoxitin and oxacillin indicating they were methicillin resistant *Staphylococcus aureus* strains (Table 1). This study has demonstrated that the dogs sampled carried CoP MRSA in the exposed wounds. The characterization of staphylococcal species that colonize pets, especially in exposed wounds, is important for maintaining animal health and to minimize the risk of transmission to owners.

Furthermore, the antimicrobial susceptibility patterns of the 13 CoP MRSA showed that only 1 isolate showed resistance to 2 (8 %) antimicrobials, 8 (62 %) to 3 antimicrobials, 3 (23 %) were resistant to 4 antimicrobials, and 1 (8 %) to 5 antimicrobials tested in various disc concentrations. Magiorakos et al., [9] have proposed that isolates showing resistance to three or more antimicrobial classes interpreted by clinical breakpoints were classified as multidrug-resistant (MDR). This signifies that 12 CoP MRSA isolated in this study were multidrug resistant. In the same vein, only 7 (63.63 %) isolates were susceptible to 2 antimicrobials, 4 (36.36 %) were susceptible to 2 antimicrobial and 1 (0.16 %) had intermediate susceptibility to 1 antimicrobial.

This study shows that antimicrobial resistance occurs in coagulase-positive staphylococci cultured from open wounds in dogs in the study area. We could not find any literature on any collection of isolates which represents CoP MRSA in dogs in Nigeria. A study by Chah et al [3] had only reported coagulase negative (CoN) methicillin-resistant strains in dogs in Nsukka, Nigeria.

CONCLUSION

The study is of clinical significance since without the knowledge of antibiotics resistance, especially methicillin

resistance in *Staphylococcus aureus*, treatment of wounds in pet dogs would be a recurring issue due to resistance of the organisms involved. It is of a public health importance, as the spread of MRSA to humans is palpable and it would be an issue in the medical settings as well as it could lead to livestock acquired MRSA (LA-MRSA). Acquired MRSA in hospitals, humans and livestock has been a global challenge. We encourage continuous surveillance for MRSA to give a broader picture of the problem and a future way forward to address this recurring veterinary and medical risk.

DATA AVAILABILITY STATEMENT

The raw data of this article will be made available by the authors, without undue reservation.

ETHICAL APPROVAL

Even though the sample collection procedure was not invasive, approval was sought and granted by the animal care and use committee (ACUC) of the National Veterinary Research Institute, Vom, Nigeria. Similarly, informed consent of the dog owners was obtained prior to sample collection. The reason for the research was fully explained to the clients.

CONFLICT OF INTEREST

We declare that authors have no competing interests.

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GENERATIVE AI STATEMENT

The authors declare that no Gen AI was used in the creation of this manuscript.

AUTHORS CONTRIBUTIONS

KAA: Conceived the idea, wrote the proposal and manuscript.

TAD: Samples collection.

IL: Samples processing.

MM: Approved the manuscript and permission to publish.

All authors have read and proofread the manuscript and agreed for publication.

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