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EVIDENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN PET AND STRAY DOGS WITHIN SOKOTO METROPOLIS, NIGERIA

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ABSTRACT

Methicillin Resistant *Staphylococcus aureus* (MRSA) is an important zoonotic pathogen capable of causing life threatening disease conditions in humans. A cross-sectional study was conducted to investigate the presence of MRSA in both pet and stray dogs within the Sokoto metropolis. A total of 100 oral swabs comprising 50 each from pet and stray dogs were collected and analyzed using routine bacteriological cultures and molecular identifications. Out of the 100 samples examined, 15 % (15/100) were positive for MRSA with varying detection rates of 9/50 (18 %) and 6/50 (12 %) for the pet and stray dogs respectively. The statistical analysis showed no significant association between the occurrence of MRSA and the dogs (P = 0.401). The study revealed the presence of MRSA in dogs within the Sokoto metropolis, which presents health risks to pet dog owners, veterinarians, dog catchers and other individuals who may come into close contact with these dogs.

Key words: antibiotics; dogs; Nigeria; Sokoto

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global public health problem, associated with considerable morbidity and mortality [29]. It is a well-known and widespread pathogen that has the ability to cause a wide spectrum of clinical diseases in both humans and other animals [7]. MRSA infections have been on the increase in the past few decades and as a result, the community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) has been reported in many middle- and low-income countries. Although the origin of the CA-MRSA is still unclear, several clones of the pathogen have been reported to be rapidly spreading within communities and healthcare settings in many countries. Individuals having close contact with animals have been shown to be at a higher risk of contracting livestock acquired MRSA [25, 33].

Methicillin resistance is conferred by the *mecA* gene, which encodes an aberrant penicillin-binding protein (PBP2a) with decreased affinity for β-lactam antibiotics, so that PBP2a are able to catalyze the transpeptidation reaction required for peptidoglycan cross-linking thereby, ena-

bling cell wall synthesis in the presence of high concentrations of β-lactams which otherwise would inhibit the endogenous PBPs; thus, conferring resistance to all β-lactam antibiotics [3, 14].

There is a sizeable population of stray dogs in Nigeria that serve as reservoirs of many environmental pathogens and potential source of infections to pet dogs that are let outdoors unmonitored. Both dogs share the same outside environment and often come into contact with each other with or without the knowledge of the owners. Thus, there is potential interchange of pathogens between the dogs which could consequently extend to the pet owners or persons in close contact with dogs such as admirers and veterinarians.

MRSA strains carry a diverse and transmissible genetic element designated as MRSA colonization in dogs which have been extensively studied in most parts of the world with varying prevalence rates of 0—6% [23]. However, despite reports of the pathogen in humans [11] and the unethical use of antibiotics by veterinary quacks and animal owners, there is very little information about the presence of MRSA in dogs in Nigeria.

MATERIALS AND METHODS

Ethical approval

All experiments were done in accordance with the guidelines to the use and care of animals of the Research Ethics Committee of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria (UDUS/FAREC/01/2019).

Study design and sample collection

A cross-sectional study was conducted between March and November, 2019. With the consent of the owners, oral swabs were collected from pet dogs across the Sokoto metropolis and from stray dogs captured and stationed at the Army barracks in Mammy Market, Sokoto. A total of one hundred samples comprising 50 each from both pet and stray dogs were randomly collected using a swab stick and transported in Brain Heart Infusion Broth (BHIB) to the Bacterial Zoonoses Laboratory, Usmanu Danfodiyo University, Sokoto, Nigeria.

Bacteriological culture and isolation

All swab samples were incubated in the transport medium (brain heart infusion broth) for 24 hours at 37°C, before being inoculated onto blood agar (containing 5% sheep blood) and incubated aerobically at 37°C for 24 hours. Colonies that appeared relatively small, circular, convex, smooth, and grayish to white and displayed light to golden yellow pigment were presumed to be *Staphylococcus aureus* and were sub-cultured onto Mannitol Salt agar (Oxoid, Basingstoke, UK) and incubated aerobically at 37°C for 24 hours. Presumptive colonies of *Staphylococcus aureus* were identified as small, smooth, golden, shiny, convex, and with golden yellow zones. The colonies were then preserved by sub-culturing onto nutrient agar (Oxoid) slant and incubated at 37°C for 12 hours. The presumptive *Staphylococcus aureus* isolates were further confirmed using a biochemical test including catalase and coagulase tests as described by Ochei, Kohlhatkar [28] and Cheesbrough [5].

Phenotypic and genotypic characterization of MRSA

To determine phenotypic methicillin resistance, overnight fresh cultures of *S. aureus* were inoculated onto a freshly prepared Oxacillin Resistance Screening Agar Base using the appropriate supplement (ORSAB; Oxoid®). Colonies that appeared intense blue on ORSAB were phenotypically presumed to be MRSA.

For genotypic characterization, the genomic DNA of the presumptive MRSA isolates were extracted using a boiling method with slight modifications as described by Chen et al. [6]. Briefly, a loop-full suspension of overnight grown cultures on nutrient agar plate was transferred into a 1.5 ml micro-centrifuge tube containing 100 µl of sterile distilled water. The suspension was first incubated at room temperature for 5 minutes, and then heat treated to 96 °C for 10 minutes. This was followed by centrifugation at 12,000 g for 5 minutes. The supernatant (containing the DNA) was then collected in a new 1.5 µl tube and kept at 4 °C until use.

PCR detection of the *mecA* gene

The DNA templates of all the positive ORSAB *S. aureus* isolates were subjected to PCR for the detection of the 163 bp fragment of the *mecA* gene using that described by Mehrotra [24] (Table 1). The PCR assay was performed in a 25 µl reaction mixture containing 3 µl

of nuclease free water, 5 µl (0.5 µg) of the DNA template, 1 µl (0.2 µM) of each forward and reverse primer, 2.5 µl of coral red dye load and 12.5 µl (100 µM) of the Master mix (Qiagen). The amplification protocol consisted of 35 cycles of amplification with an initial denaturation of 94 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, an extension at 72 °C for 1 min and then final extension at 72 °C for 7 min. Confirmed *mecA* through sequencing in the lab was used as a positive control, while nuclease free water was used in place of DNA template as a negative control. The PCR products were visualized after electrophoresis for 45 min at 90 volts in 1 % agarose gel. The amplicons were then viewed under a UV trans-illuminator (Biorad Gel Doc XR System w/Universal Hood II).

Data analysis

The results presented in tables and percentages were computed using Microsoft Excel software version 2010. Chi-squared statistics were performed using SPSS v23 to determine the possible association between MRSA colonization and the dog type.

Table 1. Oligonucleotide sequences for the detection of *mecA* gene

Primer name	Primer sequence [5′ to 3′]	Band size [bp]
*mecA*1_forward	AAAATCGATGGTAAAGTTGGC	163
*mecA*2_reverse	AGTTCTGCAGTACCGGATTTGC	

RESULTS

Of the 100 oral swabs examined, 44 % (44/100) were positive for *Staphylococcus aureus* based on culture, and biochemical characterization. Pet dogs had the highest

Table 2. Distribution of *Staphylococcus aureus* in pet and stray dogs

Status	Pet dogs	Stray dogs	Total
Positive	26	18	44
Negative	24	32	56
Total	**50**	**50**	**100**

P = 0.796; 95 % CI: 0.117—16.444

Table 3. Distribution of methicillin resistant *Staphylococcus aureus* in pet and stray dogs

Status	Pet dogs	Stray dogs	Total
Positive	9	6	15
Negative	41	44	85
Total	**50**	**50**	**100**

χ^2 = 0.706; p = 0.401, 95 % CI: 0.203—1.899

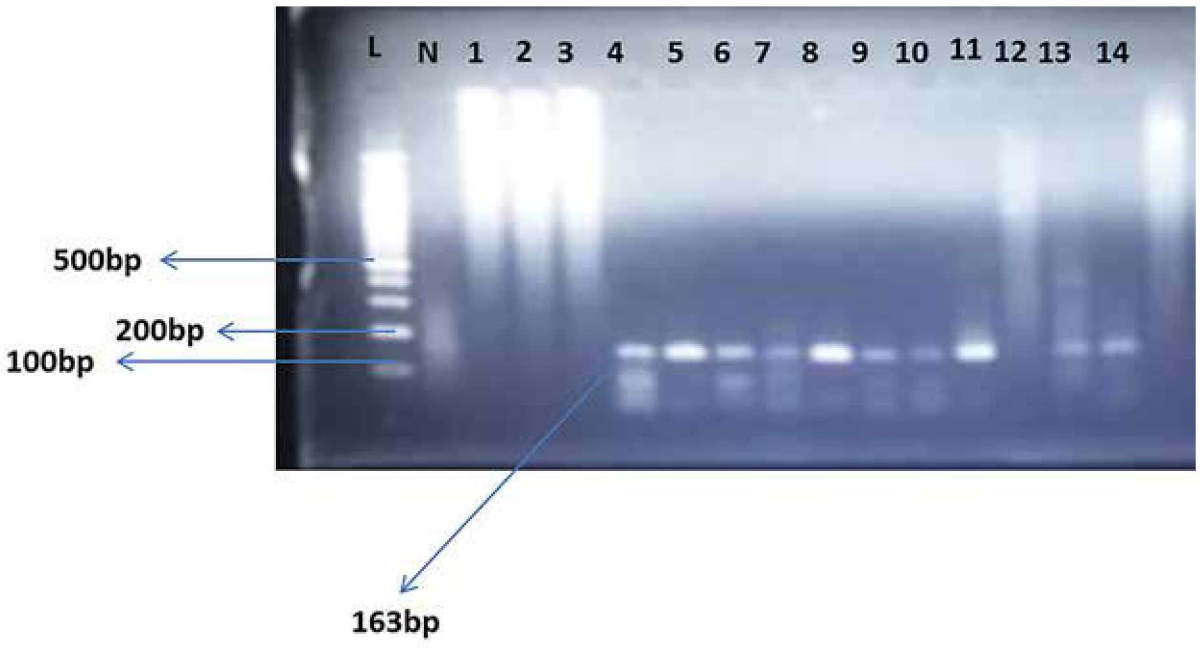

Fig. 1. Gel image of 163 bp gene fragment of the *mecA* gene using 100 bp molecular weight DNA ladder

prevalence with 52% (26/50), while stray dogs had 36% (18/50). There was no statistically significant association (P = 0.796) between the recovery rate of *S. aureus* and dog type (Table 2). The PCR detection of *mecA* gene showed that 15% (15/100) of MRSA isolates from dogs were positive (Figure 1). This comprised 18% (9/50) from pet dogs and 12% (6/50) from stray dogs. There was no statistically significant association (P = 0.40) between the recovery rate of MRSA and dog type (Table 3).

DISCUSSION

Methicillin resistance has increasingly been reported in staphylococcal isolates from canines in several countries [2, 16, 17, 36]. In the Sokoto state, the association between pet animals and humans has changed in the last few years. The number of owned dogs has dramatically increased in the society which has made dogs in closer contact with humans. This increase in acceptance of dogs as pets, partly due to the rising security challenges in the State and country at large, has increased the frequency of close contact between dogs and humans. Thus, it increases the risk of disease transmission between pets and humans. In this study, the overall oral carriage of MRSA from dogs was 15% (15/100), which was higher than the reports by Sasaki etal. [32], Abbott et al. [1], Kottler et al. [19], Penna et al. [30] Loeffler et al. [22] and Chah et al. [4] who, respectively, recorded 1.7%, 1.1%, 3.3%, 3.4%, 9.0% and 12.8% of MRSA in the dogs in the Ireland, UK, the US, and Nigeria, respectively.

This discrepancy is likely due to: differences in the breeds of dogs utilized in this study, indiscriminate antibiotic medication, which is prevalent in impoverished nations, harsher environmental difficulties often found in the study area, and malnutrition, which causes stress and impairs a dog's immune system. According to Kutdang et al. [20] findings, stray dogs and pet dogs raised in underdeveloped nations are more exposed to the outside environment and therefore, have a higher risk of catching diseases than domestic dogs. Floras et al. [10] added to the findings of this investigation by reporting on an increased rate of MRSA identification in dogs in Canada.

Contrarily, higher MRSA carriage of 67.5% and 51.1% have been reported by Vinczee et al. [35] and Iverson et al. [15], respectively. The non-significant statistical association (P = 0.401) observed between the presence of MRSA and dog type showed that both pet and stray dogs harbor the pathogen without preference associated with type of living condition. Pet dogs could acquire MRSA through contact with humans and other dogs [9]. However, the presence of the pathogen in stray dogs indicates possible contact between pet and stray dogs, which could be directly or indirectly through sharing the same environment [34]. Although, isolation of *S. aureus* is not uncommon in canine infection, MRSA isolation is of great concern as it becomes more prevalent among dogs in different regions. MRSA isolation among dogs has great public health implications because of the high risk of human infections following close contact with dogs [15]. Several studies have described potential transmission of MRSA strains between pet dogs and humans [9, 34]. However, to the best of our knowledge, this is the first study on the occurrence of MRSA in stray dogs in Nigeria. The presence of the pathogen in this type of dogs have serious public health consequences as they could serve as reservoir and sources of infection for pet dogs and other animals, including livestock that could have close contact with these dogs.

Several studies have suggested that dogs may be one of the gates through which MRSA found its way outside healthcare facilities leading to possible community transmission of such pathogen among animal and human populations [8, 21]. In agreement to this assertion, several studies in different countries have reported MRSA strains from dogs to be indistinguishable from the human strains [7, 26].

The prevalence of *S. aureus* and MRSA in the nasal cavity of dogs was extremely high. These findings show that dogs are more likely to get contaminated through their nostrils. Contaminations can also occur as a result of scavenging of death corpses from backyard poultry farms that have been exposed to antibiotics. These data, along with those of Rich, Roberts [31] and Khanna et al. [18], show that nasal sampling is a better way to detect MRSA colonization in animals. A single case of MRSA discovery from nasal swab samples of 255 dogs, rather than from the throat and skin of the same animals was confirmed by Rich and Roberts [31].

The current study's PCR investigation indicated the presence of the *mecA* gene at 163 bp in 15 of the 20 isolates that were phenotypically MRSA positive. When it comes to MRSA detection and characterization, the pres-

ence of the *mecA* gene is the gold standard [11]. According to studies, the existence of the *mecA* gene in animals and animal products supports this claim [12, 27, 37].

Some of the phenotypic MRSA positive isolates did not display *mecA* on amplification, which could imply the occurrence of *mecA* PCR negative MRSA isolates in Sokoto, according to the PCR study. Garcia-Alvarez et al. [13] discovered a novel allele of the *mecA* gene encoding an alternative penicillin binding protein that mediates methicillin resistance in bovine *S. aureus* isolates and humans in the UK, Denmark, and Germany who were Methicillin-resistant but *mecA* PCR negative in a previous study. These unique *mecA* alleles (*mecA* LGA251) exhibit a nucleotide similarity of 70 % with the archetypal *mecA* gene. Furthermore, the findings suggest that an additional *mecA* allele is circulating in the environment, which could be acquired by *S. aureus* and result in the development of a new MRSA strain.

CONCLUSIONS

This study showed the presence of MRSA in apparently healthy dogs (stray and pets) within the Sokoto metropolis of Nigeria. The findings also indicated that both pet and stray dogs harbour the pathogen with a potential risk of infecting individuals in close contact. There is the need to educate pet owners and dog catchers on the potential zoonotic transmission of MRSA from dogs and the need to take appropriate preventive measures.

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