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Antimicrobial susceptibilities of methicillin resistant *Staphylococcus Aureus* (MRSA) isolates recovered from perineum and nasal mucosa swab samples of dogs in Niger

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ABSTRACT

Methicillin Resistant Staphylococcus aureus (MRSA) is an important opportunistic pathogens of dogs, other domestic animals and humans. The proximity between humans, livestock and antimicrobial use in animals facilitates the emergence and spread of MRSA. In this study, 400 swab samples taken from the perineum and nasal mucosa of dogs. Swab samples were inoculated on 5% blood agar (Sigma® Switzerland) for 24 hours of aerobic incubation at 37 °C, growth with yellowish-white colonies with smooth, slightly raised surfaces were further gram stain and biochemical test of Coagulase and catalase tests was performed. Positive isolates were then inoculated mannitol salt agar (MSA, Oxoid) and incubated for 24 hours at 37 °C and further confirmed using Oxacillin Resistance Screening Basal (ORSAB) medium (Oxoid, Basingstoke, United Kingdom). Antibiotics susceptibility testing was determined using the Kirby Bauer's disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guideline and positive S. aureus were inoculated on Mueller-Hilton Agar (Oxoid) plates and Vanier callipers were used to measure the zones of inhibition in millimetres (mm). Out of the 200 samples each from the nasal mucosa and perineum, 55%(72) and 39.5% (49) were positive for MRSA respectively. Out of the 206 male and 194 female dog sampled, 57.0% (69) and 43.0%(52) were positive for MRSA respectively. Nigerian indigenous breed (Mongrel) has the highest proportion of MRSA isolates with 48.8% (59) while Golden retriever has the least proportion of MRSA isolates with 0.8% (1). Most of the MRSA isolates were resistant to oxacillin, cefoxitin, tetracycline, erythromycin and cephazolin but susceptibile to gentamycin, ciprofloxacin and chloramphenicol.

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Introduction

Staphylococcus aureus (*S. aureus*) belongs to the members of the microbiota of human body, usually isolated from the upper respiratory tract and the skin. Although they can be opportunistic organism, resulting in skin and respiratory infections (Oh et al., 2020). Methicillin-resistant *S. aureus* (MRSA) has raised public health concerned all over the world (Deleo and Chambers, 2009). About 20% to 30% of humans are carriers of *S. aureus*, which are the normal microbiota of the skin and nasal mucosa (Tong et al., 2015). They are frequently distributed in nosocomial infections and wound infections after surgery.

The close interactions between humans, livestock and antimicrobial use in animals favors the emergence and spread of MRSA (Bouchami et al., 2020; Lawal et al., 2021). Since the year 2000, there have been the emergence of multi drug resistant as an opportunistic organism in companion dogs, dog's owners, veterinary hospitals and veterinary clinicians (Sweeney et al., 2018). Its common in dog population of about 20% (Gomez-Sanz et al.,2013; Afshar et al., 2023). MRSA infection has given rise to serious public health

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concerned because infection rate increases every year (Decline et al., 2020). The irrational use of antibiotics has given rise to the emergence of resistance to antibiotics due to *S. aureus* refer to as methicillin-resistance *S. aureus* (MRSA) (Decline et al., 2020).Transmission between animal to human usually occur because pet animals are regarded as family members, resulting in to close contacts between humans and pets which cause bacterial transmission dynamics (Decline et al., 2020). This is a serious public health problem because human MRSA can be transmitted to pets, and there after pets can also serve as source of infections to humans, as such pet animals can be regarded as reservoir for spreading infections to humans when they come in contact (Reddy et al., 2016; Findik et al., 2018; Decline et al., 2020). Human infections with MRSA methicillin resistant *staphylococcus aureus* have been documented in several parts of Nigeria (Aliyu et al., 2022). Pets especially dogs are kept at home for security reasons due to increase urbanization and raise in antisocial behaviors and crimes. These days relationships bond between humans and pet animals is on the increase and this could facilitate the spread of infectious or zoonotic organisms to humans (Audu et al., 2022). Due to continuous changes in the prevalence and epidemiology of MRSA, and the discovery of new strains of MRSA in Nigeria. The present study was designed to assess the prevalence of MRSA, antibiotic susceptibility profile and potential risk factors associated with dogs in Gombe State, Nigeria.

Materials and Methods

Study area: Gombe state is located in the North east geopolitical zone of Nigeria, the state covers an area of 20, 265km2 and located between latitudes 9°301N and 12°301E and longitude 8°451 and 11°451E.The state has a total of eleven local Government areas (LGAs) and 114 wards. The LGA are Akko, Balanga, Billiri, Dukku, Funakaye, Gombe, Kaltungo, Kwami, Nafada,, Shongom and Yamaltu/Deba (Wikipedia, 2006).

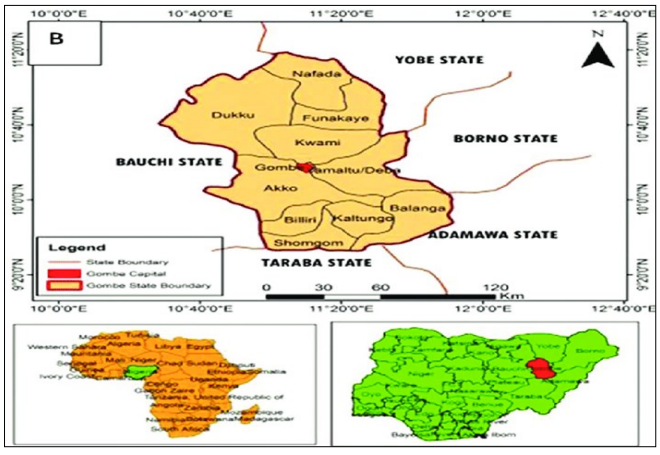

Figure 1. Map of Gombe State showing the study area

Sampling procedure and study population: A cross-sectional study was conducted. Six LGAs (Kwami, Gombe, Yamaltu Deba, Akko, Billiri and Kaltungo) out of the eleven LGAs were selected randomly (two from each senatorial zone). Facilities surveyed were the State and Private Veterinary clinics, households and dog markets. The quantity of samples gathered from every one of the six LGAs was determined by accessibility and availability. A systematic random sampling technique was used to choose individual canines, with one dog out of every two that were sighted being chosen (Pfeiffer, 2002). Using the 50% prevalence at 95% Confidence level and the Thrusfield (2005) formula, the sample size was calculated. For accuracy, the 384 sample size that was estimated was raised to 400.

Sample collection and processing: Dogs were sampled with the owners' permission. Every dog had one nostril opened, and a cotton-tipped sterile swab (Everson Industries Limited, Nigeria) was used to obtain a sample from the nasal mucosa, which was then quickly placed into an aseptic tube. The perianal area is rolled with a fresh, sterile cotton-tipped swab, which is then promptly placed back into its tube. The dates of collection and an identifying number were written on the labels of the tubes in accordance with laboratory protocol (Cheesbrough, 2010). The samples were transported to the Bacterial zoonosis lab of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU) Zaria on an ice pack for immediate processing. Using recognized microbiological techniques, such as colonial morphology, gram stained features, catalase and coagulase tests, the organisms were isolated aseptically (Cheesbrough, 2010). Oxacillin Resistance Screening Agar Base (ORSAB) was used to detect MRSA. The disk diffusion method was used to carry out the anti-microbial sensitivity test.

Isolation and identification: The manufacturer's instructions were followed to make an enriched solid medium containing 5% blood agar (Sigma® Switzerland) and inoculate the swabs into it (Cheesbrough, 2010). To obtain distinct colonies, the inoculums were streaked using a sterile wire loop. After 24 hours of aerobic incubation at 37°C, the infected plates were checked for yellowish-white colonies with smooth, slightly raised surfaces. While some positive colonies were non-hemolytic, others had entire zones of hemolysis.

Colony morphology: The samples that were taken underwent gram staining in order to determine the staphylococci based on their gram reaction. Coagulase and catalase tests were performed on samples that were organized into clusters resembling grapes.

Mannitol salt agar (MSA, Oxoid) is a selective medium for S. aureus. The plates were streaked with the positive isolates and then incubated for 24 hours at 37°C in an aerobic environment using mannitol salt agar (MSA, Oxoid). It was assumed that S. aureus was the source of the yellowish colonies that appeared on MSA.

Oxacillin resistance screening agar base (ORSAB): Methicillin resistance was presumably determined using ORSAB agar (OXOID), a commercial medium. ORSAB was made in compliance with the manufacturer's guidelines. MRSA can be screened using ORSAB, a medium that is nutrient-rich, selective, and contains growth-promoting agents for microbes. Mannitol and aniline blue are added to the medium together with a high concentration of salt and lithium chloride to inhibit non-staphylococcal growth and detect mannitol fermentation. The antibiotics included in the ORSAB selective supplement are polymyxin B to limit the growth of other bacteria that can tolerate such a high concentration of salt e.g. Proteus spp. and oxacillin at 2 mg/L to inhibit methicillin-sensitive *S. aureus*. The characteristic blue color of MRSA colonies against a colorless background made it easier to identify the bacteria.

Antibiotic susceptibility testing: Using the Bauer-Kirby approach, the antibiotic susceptibility of MRSA isolates was ascertained (Bauer et al., 1966). Utilizing the commercially produced disk (Oxoid, UK) that has an established antibiotic concentration. Three to four millilitres of sterile normal saline were used to emulsify freshly sub-cultured MRSA and well-isolated colonies from ORSAB plates. The suspension's turbidity was corrected to 0.5 McFarland, which is the standard equivalent (CLSI, 2019). A sterile cotton swab stick was dipped into the suspension of Mueller-Hinton agar medium. By applying pressure and spinning the swab against the tube's side above the suspension, extra fluid was eliminated. In order to guarantee even dispersion, the plate was rotated by about 60° after the swab was evenly streaked over the medium's surface in three directions on the dried Mueller-Hinton agar surface (Benkova et al., 2020). After being distributed into each inoculation plate, five antimicrobial discs were incubated for twenty-four hours at 35°C at a time. Vanier callipers were used to measure the zones of inhibition in millimetres (mm). The Clinical and Laboratory Standard Institutes (CLSI) criteria were utilized to interpret the sizes of the zones of inhibition. The following 10 antibiotics were tested; ciprofloxacin (CIP) 5 ug; erythromycin (ERY) 15 ug; gentamycin (GEN) 5 ug; tetracycline (TET) 30 ug; Clindamycin (DA) 2 ug; chloramphenicol (CHL) 30 ug; sulfamethoxazole/trimethoprim (STX) 25 ug; cefoxitin (CFX) 30 ug; cefazolin (CZO) 30 ug; oxacillin (OXA) 1 ug. Growth inside the zone of inhibition was thought to be suggestive of methicillin resistance for the interpretation of susceptibility toward the oxacillin disc. A diameter of inhibition zones of ≤10, 11–12, and ≥13 by 1 ug of oxacillin is classified as susceptible (S), intermediate (I), or resistant (R) to oxacillin correspondingly, based on the classification criteria provided by (CLSI, 2019). Regarding cefoxitin discs, staphylococci classified as either sensitive or resistant to oxacillin are indicated by inhibitory zone diameters of ≥24 and ≥25 mm, respectively. The cefoxitin disc diffusion test does not classify staphylococci into any intermediate category (CLSI, 2019).

Statistical analysis: Data generated were presented as frequency and percentages using descriptive statistics. Graph pad prism version 5 was used to analyzed the data generated. Chi-square/Fisher's exact test was employed to determine the association between MRSA colonization and site of isolation as well as sex of the dogs, values of $p<0.05$ were considered significant. The prevalence was calculated for all data as the number of MRSA colonized individuals divided by the number of sampled dogs in the category and was expressed in percentage by multiplying by 100.

Results

A total of 400 swab samples were collected from different breeds of dogs. Out of the 200 samples each from the nasal mucosa and perineum, 55%(72) and 39.5%(49) were positive for MRSA respectively (Table 1). There was no statistically significant association between MRSA colonization and site of isolation ($P>0.05$). Out of the 206 male and 194 female dogs sampled, 57.0% (69) and 43, 0%(52) were positive for MRSA respectively (Table 2). There was no statistically significant association between MRSA colonization and Sex of the dogs ($P>0.05$). Nigerian indigenous breed (Mongrel) has the highest proportion of MRSA positive isolates with 48.8%(59) while Golden retriever has the least proportion of MRSA positive isolates with 0.8%(1) (Table 3). Most of the MRSA positive isolates were resistant to oxacillin, oefoxitin, tetracycline, erythromycin and cephazolin. However, susceptibility to gentamycin, ciprofloxacin and chloramphenicol was remarkable (Table 4). Multidrug resistant pattern of the isolates was also evaluated and shows varying levels of resistance to multiple class of antimicrobials (Table 5)

Table 1. Prevalence of MRSA isolated from dogs according to site of isolation in Gombe State

Sites	Number of samples	*S. aureus* (%)	MRSA (%)
Nasal mucosa	200	129 (64)	72 (55.8)
Perineum	200	124 (62)	49 (39.5)
Total	400	253 (63.3)	121 (47.8)

P-value = 0.0667, 95% confidence interval (C. I.) = 0.4505-1.028, odds ratio (OR) = 0.6806

Table 2. Prevalence of MRSA isolated from dogs according to sex in Gombe State

Sex	Number of Samples	*S. aureus* (%)	MRSA (%)
Male	206	137 (45.8)	69 (57.0)
Female	194	116 (54.2	52 (43.0)
Total	400	253 (63.2)	121 (47.8)

P-value= 0.3002, 95% confidence interval (C. I.) = 0.5311-1.206, odds ratio (OR) = 0.8002

Table 3. Breed distribution of S. aureus and MRSA in dogs in Gombe State

Dog breeds	*S. aureus* isolates (%)	MRSA positives (%)
Mongrel	101 (39.9)	59 (48.8)
Alsatian	31 (12.3)	16 (13.2)
Rottweiler	18 (7.1)	5 (4.1)
Boerboel	13 (5.1)	2 (1.7)
Golden retriever	6 (2.4)	1 (0.8)
Caucasian	28 (11.1)	13 (10.7)
Lhasa apso	11 (4.3)	3 (2.5)
Alsatian/Mongrel cross	25 (9.9)	14 (11.6)
Caucasian/Mongrel cross	20 (7.9)	8 (6.6)
Total	256	121

Table 4. Antibiotic susceptibility pattern of MRSA isolated from dogs in Gombe State

Antibiotic	Resistant no. of isolates (%)	Intermediate resistant no. of isolates (%)	Susceptible resistant no. of isolates (%)
Chloramphenicol	19 (15.7)	27 (22.3)	75 (62.0)
Cephazolin	84 (69.4)	21 (17.4)	16 (13.2)
Oxacillin	121 (100)	0 (0)	0 (0)
Ciprofloxacin	1 (0.8)	13 (10.7)	107 (88.4)
Sulphamethaxazole/ Trimethoprim	6 (5.0)	21 (17.4)	94 (77.7)
Erythromycin	86 (71.1)	25 (20.7)	10 (8.3)
Gentamycin	0 (0)	1 (0.8)	120 (99.2)
Tetracycline	104 (86.0)	11 (9.1)	6 (5.0)
Cefoxitin	121 (100)	0 (0)	0 (0)
Clindamycin	34 (28.1)	39 (32.2)	48 (39.6)

Table 5. Multidrug resistant patterns observed in MRSA positive isolates from dogs in Gombe State

Number of antibiotics class	Resistance patterns	Number of bacterial isolates
3	CFX + CHL + CIP	1
3	OXA + CFX + DA	6
3	OXA + CFX + CZO	3
3	ERY + OXA + STX	5
3	OXA + TET + CFX	13
4	DA + OXA + CFX + TET	10
5	CZO + OXA + ERY + TET + CFX	30
5	CZO + OXA + ERY + TET + CFX	28
6	CZO + OXA + STX + ERY + TET + CFX	5
7	CZO + OXA + ERY + TET + CFX + DA + CHL	18

CIP= Ciprofloxacin; ERY= Erythromycin; GEN= Gentamycin; TET= Tetracycline; DA= Clindamycin; CHL= Chloramphenicol; STX= Sulfamethoxazole/trimethoprim; CFX= Cefoxitin; CZO= Cefazolin; OXA= Oxacillin

Discussions

Methicillin resistance has increasingly been reported in staphylococcal isolates from canines in several countries (Jang, et al., 2014; Ishihara et al., 2014; Decline et al., 2020; Afshar et al., 2023) including Nigeria (Yakubu et al., 2022). In this study, the overall prevalence of MRSA in dogs was 47.8%. This was greater than 36.9 % and 15 % (15/100) of MRSA that were reported in Maiduguri and Sokoto, Nigeria, respectively, by Mustapha et al. (2016) and Yakubu et al. (2022). It also surpassed the reports by Abbott et al. (2010), Kottler et al. (2010), Chah et al. (2014), and Penna et al. (2021), who recorded 1.1%, 3.3%, 12.8 %, and 3.4% of MRSA in dogs in Ireland, the United States, Nigeria, and Brazil, respectively.' This might probably be attributed to variation in the breeds of dogs used in this finding, indiscriminate antibiotic therapy, harsher environmental challenges and malnutrition, 'which weakens the immune system and induces stress in dogs' (Yakubu et al., 2022). Contrarily, higher MRSA carriage of 67.5 % and 51.1 %have been reported by Vincze et al. 2014 and Iverson et al. 2015, respectively.

The prevalence of MRSA in the nasal cavity with the rate of 55% was found to be higher than that of the perineum with the rate of 39.5%. This result in the current was in line with the Mustapha et al (2016)who reported the MRSA the rates of 50% and 30% determined in the nasal cavity and perineum, respectively. The prevalence of S. aureus and MRSA in the nasal cavity of dogs was determined to be extremely high. These findings showed that dogs are more likely to get contaminated through their nostrils and nasal sampling is a better way to detect MRSA Rich and Roberts, (2004). 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, (2004). The prevalence of MRSA in male dogs was 57% as against the 43% in the female. Breed distributions shows that Nigerian indigenous breed (Mongrel) has the highest proportion of MRSA positive isolates with 48.8% (59) whereas Golden retriever (exotic breed) has the least proportion of MRSA positive isolates with 0.8%(1). According to Kutdang et al. (2010) findings, native dogs raised in developing nations was more exposed to the outside environment and therefore, have a higher risk of contracting diseases than the exotic breeds of dogs.

The results of susceptibility patterns of MRSA to antibiotics showed a decreasing trend of resistance in the order; Oxacillin (100%), Cefoxitin (100%), Tetracycline (86%), Erythromycin (71.1%), Cephazolin (69.4%), Clindamycin (28.1), Chloramphenicol (15.7%), Sulphamethaxazole/Trimethoprim (5%), Ciprofloxacin (0.8%) and Gentamycin (0%). This may be as a result of the fact that, they are commonly used by veterinarians and dog owners. This study showed that commonly used antibiotics in the study area such as Tetracycline is no longer reliable in treating staphylococcal infections in this region as clearly seen in the 86% resistance of the isolates to this antibiotic. This result agrees with the results of Mustapha et al., (2016) in Maiduguri, Nigeria which shows high resistance to Tetracycline (73.4%). Further, the high resistance to the antibiotics can be explained in terms of their widespread and reckless use, as well as their affordability (Zedan et al., 2023). High percentage susceptibility of MRSA isolates Gentamycin (99.2 %), Ciprofloxacin (88.4%), Sulphamethaxazole/ Trimethoprim (77.7 %) and Chloramphenicol (62 %) was found to be in line with Yaser et al (2015)'s results. Gentamycin was the most sensitive among all the antibiotics used in this study. The increased sensitivity to gentamycin may be due to the fact that dog owners and veterinary professionals in the study area do not frequently use gentamycin for treatment. An acquired non-susceptibility to at least one agent in three or more antimicrobial categories is known as multidrug resistance, or MDR (Magiorakos et al., 2012). High rate of MDR was observed among the MRSA isolates as most of them were resistant to more than two classes of antimicrobial agents. Although the focus of the study has been on methicillin resistance, S. aureus may be resistant to any antimicrobial treatment. It might jeopardize the efficacious management of S. aureus infections in both humans and animals (Crespo□Piazuelo and Lawlor, 2021). Therefore, S. aureus that is resistant to antibiotics can live in animal reservoirs and spread to humans and other animals through contact. For improved management of Staphylococcal infection in humans and animals, it's critical to monitor for the emergence of resistant infections in animal reservoirs (Zedan et al., 2023).

Conclusion

This study established the prevalence of MRSA in dogs in Gombe State as 47.8 %. Gentamycin was the most sensitive among all the antimicrobials tested and therefore the drug of choice in the study area. Most of the isolates shows resistance to multiple class of antimicrobials, hence the need for proper policies and program on antimicrobial use by the regulatory authority.

Ethics Committee Approval: This research did not involve invasive procedure as such an ethics committee approval is not required.

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