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Phenotypic Detection of Methicillin-Resistant *Staphylococcus aureus* in Clinical Samples of Dogs and Their Owners in Buwaya, Gonin-gora, Kaduna State, Nigeria

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Abstract

Methicillin-Resistant Staphylococcus aureus (MRSA) are multidrug resistant pathogen of public health concern. It had been reportedly transmitted between individuals and pets in the community. In this study, MRSA was phenotypically detected among dogs and their owners in a small community (Buwaya) in Kaduna state, Nigeria. A total of 63 nasal samples were collected from both dogs and their owners, culture, microscopy and biochemical tests were used to isolate and identify Staphylococcus aureus from the samples. Detection of MRSA isolates was carried out using cefoxitin disc sensitivity testing, a surrogate marker for methicillin resistance. Kirby Bauer disc diffusion method was used for antibiotics testing.The following organisms were isolated: Staphylococcus aureus 13(20.6%), E. coli 14 (22.2%), Coagulase negative Staphylococci 21 (33.3%), Shigella spp 13 (20.6%) and Micrococcus spp 2(3.2%). All the four S. aureus isolates from the dogs were positive for methicillin resistance while 6(66%) of the owners were methicillin resistance positive which showed a total MRSA prevalence of 76.9%. Chloramphenicol (90%) was the most active of all the antibiotics tested followed by cotrimoxazole (80%), doxycycline (70%), ciprofloxacin (70%), gentamicin 60%). The highest level of resistance of the phenotypic MRSA isolates was observed to penicillin (100%), amoxicillin-clavulanic acid (100%) and tetracycline (30%). In conclusion an alarming phenotypic MRSA prevalence of 76.9% was observed among dogs and their owners, the possibility of transmission of MRSA strains between pets and humans was also observed.

Keywords: Methicillin-resistance, Staphylococcus aureus, dogs, phenotypic.

INTRODUCTION

Staphylococcus aureus are commensals that colonize the nares, axillae, vagina and pharynx or damaged skin surfaces of humans (Krismer *et al.*, 2017). It is one of the most virulent pathogens of humans and is the causative agent of a variety of deep-seated invasive and toxin-mediated infections as well as superficial infections such as boils and furunculosis (Parlet *et al.*, 2019). *S. aureus* can also colonize primates (Pantosti *et al.*, 2012; Traversa *et al.*, 2015), although *S. intermedius*has mostly been isolated from skin and mucosae of dogs and cats (Devriese *et al.*, 1985; Nagase *et al.*, 2002).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) are strains of *S. aureus*that have developed resistance to methicillin, other penicillins and other commonly prescribed antibiotics due to the presence of beta lactamase and acquisition of *mec A* gene. *S. aureus* acquires methicillin resistance by acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*) carrying *mec*A gene into chromosome.This gene encodes an altered penicillin-binding protein, PBP2a (Chrongtrakool *et al.*, 2006).

MRSA are associated with high mortality and morbidity rates, hence a major public health concern especially with the advent of community-acquired MRSA (CA-MRSA). Both animals and humans can be colonized with MRSA (Lin *et al.*, 2011). Pet animals are often in close physical contact (touching, petting, and licking) with their owners, exposing them to infection with pathogenic bacteria.

Dogs are usually colonized by MRSA strains from humans (Manian, 2003).Transmission of methicillin-susceptible *Staphylococcus aureus* and *Staphylococcus intermedius*has been reported between owners and their pets (Simoons-Smit *et al*., 2000; Tanner *et al*., 2000; Guardabassi *et al*., 2004).

MRSA had been isolated from asymptomatic dogs and mucosa carriage of MRSA isolates have been reported in dogs and other pets (Lilenbauw *et al*., 1998; van Duijkeren *et al*., 2003;Manian, 2003). They have been isolated from various skin and wound infections including abscesses, dermatitis as well as other conditions including pneumonia, rhinitis, sinusitis, otitis, bacteremia, septic arthritis, osteomyelitis, mastitis (including gangrenous mastitis) and urinary tract infections (Catry *et al*., 2010; Loffler and Lloyd, 2010).

A few data exist on the prevalence of MRSA in dogs in Nigeria; hence this study was therefore aimed at determining phenotypically the occurrence of MRSA among dogs and their owners in Buwaya community, Gonin-gora, in KadunaState, Nigeria.

MATERIALS AND METHODS

Inclusion criteria

All dogs and their handlers in Buwaya community who have not ingested any antibiotic four weeks before the commencement of this research were included in the sample collection.

Study area and sample size

Buwaya is a small community in Goningora area of Chikun Local Government area of Kaduna State Nigeria. The security challenge in this area at the time of this research necessitated the need for the use of dogs both as companions and as guards in the community. Being a small community majorly populated by farmers, all the houses were visited considering the inclusion and exclusion criteria.

Exclusion criteria

The exclusion criteria were that none of the individuals or dogs had ingested an antibiotic four (4) weeks prior to the sample collection date.

Informed consent

The households where the samples were obtained were informed prior to the sample collection and questions were asked to provide exclusion criteria.

Collection of samples

Using a sterile swab stick, nasal, rectal and skin samples of the dogs were obtained. Nasal and urine samples of the handlers/owners were also collected.

Isolation and identification of *S. aureus*

The samples were inoculated on mannitol salt agar (Liofil chem), Gram stained and the colonies viewed microscopically with characteristic cultural morphology of *S. aureus* on mannitol salt agar. The following conventional biochemical tests were carried out to further identify the *S. aureus*: catalase, coagulase, sugar fermentation on triple sugar iron agar, oxidase, urease, and deoxyribonuclease tests (Cheesbrough, 2005).

Isolation of other microorganisms

Other microorganisms aside *S. aureus* were isolated using conventional biochemical methods: triple sugar iron, citrate, oxidase, urease, methyl red, Voges Proskauer tests. The isolates were also grown on macConkey and salmonella-shigella agar (Cheesbrough, 2005).

Antibiotic Susceptibility Testing

Susceptibility to commonly prescribed antibiotics was carried out using disc diffusion method according Clinical Laboratory Standards Institute (CLSI, 2018). Susceptibility of the isolates to the following antibiotics discs were tested: Penicillin (10 units), gentamicin (10 µg), tetracycline (30 µg), sulphamethoxazole-trimethoprim (co-trimoxazole) (1.25/23.75 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), amoxicillin-clavulanic acid (30 µg) and doxycycline (30 µg). Sterile Mueller Hinton agar plates were inoculated with 0.5 McFarland standardized culture of the isolates (~1.5 x 10^8CFU/ml) using sterile swab sticks. The plates were allowed to dry, after which antibiotic discs were placed aseptically on the Mueller Hinton agar plates. Thereafter, the plates were incubated at 37˚C for 18 – 24 hours, after which the diameter zone of inhibitions were measured (in mm) and interpreted according to CLSI (2018).

Phenotypic Detection of MRSA

This was done using cefoxitin disc 30 µg, a surrogate marker for detecting methicillin resistance. All *S. aureus* isolated were screened for methicillin-resistance by following CLSI guidelines (CLSI, 2018). The plates were read after incubation at 35°C for 18 h and isolates with zone diameters ≤ 21mm were considered cefoxitin resistant.

A total number of 56 samples were collected from dogs and handlers from 5 households. The distribution of the samples collected are shown in Table 1.

RESULTS

The distribution of the samples collected are shown in Table 1.The following organisms were isolated: *Staphylococcus aureus* 13(20.6%), *E. coli*14 (22.2%), Coagulase negative *Staphylococci* 21(33.3%), *Shigella* spp 13 (20.6%) and *Micrococcus* spp 2(3.2%). This implies that 20 (31.7%) of the total bacterial isolates were from dogs while 43 (68.3%) were from dog owners (Table 2).All the 4 *S. aureus* isolates from dogs were methicillin resistant (Table 3). Out of the 5 houses where samples were collected, MRSA was detected in dog owners from 3 houses, both dogs and owners were MRSA positive in 2 houses.

The result of the antibiotic susceptibility testing showed that of all the antibiotics tested, Chloramphenicol (90%) was the most effective, followed by cotrimoxazole (80%), doxycycline (70%), ciprofloxacin (70%), and gentamicin (60%). The highest level of resistance by the MRSA isolates was observed to penicillin (100%), amoxicillin-clavulanic acid (100%) and tetracycline (30%) (Table 4). The methicillin-sensitive *S. aureus* (MSSA) were more susceptible to the antibiotics tested (Figure 1).

Table 1: Distribution of Samples Collected from 5 Households in Buwaya, Gonin-gora, Kaduna Nigeria

Houses	Dog samples (nasal, skin and rectal)	Dog owner/handler's nasal samples	Dog owner/handler's urine samples
A	3	11	8
B	3	6	6
C	3	3	0
D	3	2	0
E	3	5	0
Total	15	27	14

Table 2: Distribution of Bacterial Isolates in Relation to Dogs and their Owners

Bacterial isolates	Dogs (%)	Dog owners (%)	Total (%)
S. aureus	4 (20.0%)	9 (20.9%)	13 (20.6%)
E. coli	2 (10.0%)	12 (27.9%)	14 (22.2%)
*Shigella*spp	7 (35.0%)	6 (14.0%)	13 (20.6%)
Coagulase negative Staphylococci	7 (35.0%)	14 (32.6%)	21 (33.3%)
Micrococcus spp	0	2 (4.7%)	2 (3.2%)
Total	20 (100%)	43 (100%)	63 (100%)

Table 3: Result of Phenotypic MRSA Detection

	Dog (n = 4)	Dog owners (n = 9)	Total (n = 13)
MRSA	4 (100%)	6 (66.9%)	10 (76.9%)
MSSA	0	3 (33.3%)	3 (23.3%)

Table 4: Antibiotic Susceptibility Pattern of MRSA Isolates

Antibiotic		No of isolates (%)	
n = 10	Sensitive	Intermediate	Resistant
Amoxicillin-Clavulanate 30 µg	0	0	10 (100)
Tetracycline 30 µg	3 (30)	2 (20)	5 (50)
Gentamicin 10 µg	6 (60)	1 (10)	3 (30)
Cotrimoxazole 1.25/23.75µg	8 (80)	0	2 (20)
Chloramphenicol 30 µg	9 (90)	0	1 (10)
Ciprofloxacin 5 µg	7 (70)	0	3 (30)
Penicillin 10 units	0	0	10 (100)
Doxycycline 30 µg	7 (70)	1 (10)	2 (20)

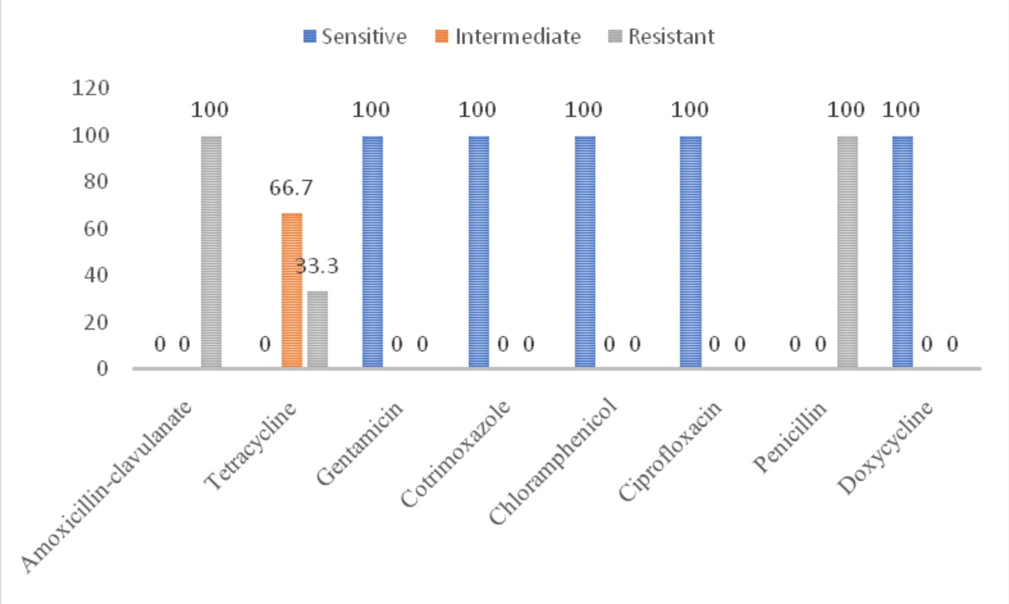

Figure 1: Percentage Antibiotic Susceptibility of MSSA Isolates

DISCUSSION

Occurrence of *S.aureus* isolates in dog owners 9 (69.2%) were found to be higher than that isolated from the dogs 4 (30.8%). *S. aureus*had been described to colonize humans more frequently than dogs; *S. pseudintermedius* is the major *Staphylococcal* specie that colonizes dogs(Kasper *et al.*, 2018; Bannoehr and Guardabassi, 2012). This was similar to previous finding where *S. aureus* isolates in dog owners were higher than that found in the dogs (Boost *et al.*, 2007, Cuny *et al.*, 2022). Isolation of *E. coli* and *Shigella* spp in this study is an indication of lack of good hygiene among the dog handlers.

There was a total of 10 (76%) phenotypic MRSA isolates, of which 4(40%) were from dogs. It was observed in few houses that both dogs and their owners were positive for MRSA. In a previous study by Yakubu *et al.*, (2022), MRSA was isolated from 9/50 (18 %) and 6/50 (12 %) of pet and stray dogs respectively while in another study by Mustapha *et al.*, (2016), 62.5% MRSA was isolated from hunting dogs in Maiduguri, Borno State, Nigeria.

The result of the antibiotic susceptibility of the MRSA isolates showed that the organisms were 100% resistant to the beta lactam antibiotics tested namely penicillin, amoxicillin-clavulanate; 50%, and 30% resistant to tetracycline and gentamicin respectively. This is not surprising since MRSA strains are known to be resistant to beta lactam antibiotics and multiple antimicrobial agents (Berger-Bachi, 1995; Hunter *et al.*, 2011), and as such treatment options are limited. MRSA can cause severe infections such as bloodstream infections, pneumonia and surgical site infections (CDC, 2019).

Studies on MRSA colonization or infection among pets had shown that both human-to-animal and animal-to-human transmission can occur (Pantosti, 2012). This was observed in this study where MRSA was isolated from both dogs and their handlers. According to Harrison *et al.*, (2014), epidemiological studies performed in different countries had shown that pets are able to exchange resistant pathogens with human populations in the same geographical area. For example, ST22(epidemic MRSA 15) in the United Kingdom, Germany, Portugal and ST59/ST239 in Chinaisolated from pet animals were found to be dominant in humans(Loeffler *et al.*, 2010; Strommenger*et al.*, 2006; Vincze *et al.*, 2013; Coelho*et al.*, 2011; Zhang*et al.*, 2011; Ho *et al.*, 2012).

The family heads of all the households where the study was conducted admitted to taking antibiotics previously without doctor's prescription. Certain households also treat their dogs with antibiotics that are used in human medicine, for example amoxicillin-clavulanic acid and tetracycline by dissolving them in water, they administer this to their dogs without consulting a veterinarian. The continuous indiscriminate exposure of dogs to antibiotics will increase the risk of development of resistance. It is therefore recommended that abuse and misuse of antibiotics by both human and pets should be avoided in order to curtail the spread of antibiotic resistant strains among pets and humans.

CONCLUSION

This study showed the presence of MRSA isolates among the dogs and their handlers in Buwaya community of Goningora, Kaduna State. All the *S. aureus* isolates from dogs in this study were methicillin-resistant. Chloramphenicol and cotrimoxazole were the most active antibiotics against the phenotypic MRSA isolates in this study. This study also showed that both human and dogs can be asymptomatic carriers of MRSA. It is therefore recommended that dogs handlers should be educated on possible transmission of infections from animals to humans and vice versa and preventive measures.

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