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RESEARCH ARTICLE

METHICILLIN-RESISTANT *Staphylococcus aureus* INFECTIONS IN DOGS IN JOS, NIGERIA

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major pathogen of damestic animals and there is concern over transmission of the organism from animals to humans. This study investigated the preliminary existence of MRSA and Methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates from dog infections in Jos and its environs. Using standard methods; the morphology, physiology and biochemical characterization of the isolates were identified. One hundred and thirty-five (135) isolates out of 200 samples were identified as *Staphylococcus aureus.* Dnase agar hydrolysis and Oxacillin agar tests were employed for, screening and susceptibility testing to identify MRSA and MSSA. Of the 135 isolates, 36(26.7%) were Methicillin-resistant *Staphylococcus aureus* and 99(73.3%) were Methicillin Susceptible *Staphylococcus aureus.* The isolation of MRSA from dog infections in the study area is of public health significance considering the medical, veterinary and zoonotic nature of the organism.

Key words: Methicillin Resistant, Susceptibility, *Staphylococcus aureus*, Dogs, Jos, Nigeria.

INTRODUCTION

The advent that of antibiotics during the 1940s and 1950s gave clinicians weapon against infections once wiped out the entire populations. The discovery and subsequent development of these antimicrobial agents revolutionized medical care worldwide. However, the dawn of the antibiotic era was quickly accompanied by the development of numerous problems including the emergence of microorganisms within resistant strains (Multidrug resistant bacteria), increased number of nosocomial infections in animals and their caretakers and community-acquired infections, arising from wide spread use of antibiotics (Synder *et al.*, 2000). *Staphylococcus aureus* is recognized as one of the most important bacteria of human and veterinary hospital environment all over the world (Leski *et al.*, 1998). *Staphylococcus aureus* is a common skin and nosopharynx commensal, a frequent causative agent of burns and wound sepsis. It produces pustules, carbuncles, furuncles and impetigo. It is the frequent causative agent of septicemia, bacteraemia, osteomyelitis, otitis and pyoderma on dogs (Emmerson, 1994). It is also a common causative agent of infections in hospitals especially in newborn babies, surgical patients, old, malnourished persons and patients with diabetes and chronic diseases (Tuo *et al*., 1995).

Methicillin–resistant *Staphylococcus aureus* was discovered in 1961 in the United Kingdom. It made its first major appearance in United States in 1981 among intravenous drug users (Carmeli *et al*., 2005). Consequently, diseases caused by Methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming increasingly more difficult. This is simply due to accumulation of new antimicrobial resistance determinants by MRSA (Henry and Elias, 1995). *Staphylococcus aureus* is a pathogen of greater concern because of its virulence (Chambers, 2005; Susan and Robert, 2006).

The control of these diseases as well as the high mortality due to *Staphylococcus aureus* was abated by penicillin in the 1940s. However, this success was short-lived as Penicillin resistant *Staphylococcus aureus* (PRSA) producing beta-lactamase quickly emerged and 90 percents of hospital-acquired *Staphylococcus aureus* were Penicillin resistant within 10 years (Susan and Robert, 2006). The beta-lactamase enzyme destroys the penicillin antibiotic by hydrolyzing the beta-lactam ring and this decreases the usefulness of the penicillin antibiotic (Susan and Robert, 2006). Methicillin a beta-lactamase insensitive beta-lactam, provided new treatment options for MPRSA infections in the late 1950s, but methicllin-resistant *Staphylococcus aureus* (MRSA) that are cross resistant to all beta-lactams soon emerged, primarily in health care environment (Susan and Robert, 2006). MRSA isolates became multi-resistant to other classes of antimicrobial. Rates of methicillin resistance increased slowly, but progressively, until the late 1990s when a dramatic surge in MRSA rates began (Carleton and Charlebosis, 2004). Methicillin resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult - to - treat infections in human and animals. It may also be called multi drug resistant *Staphylococcus aureus* or Oxacillin - resistant

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Staphylococcus aureus (Petit *et al.,* 2007). MRSA is by definition, any strain of *Staphylococcus aureus* bacteria that have developed resistance to beta-lactam antibiotics which include the Penicillins (Methicillin, Dicloxacillin, Nafcillin, Oxacillin etc.) and the Cephalosporins (Morrison *et al*., 2007).

During the past 2 decades, Methicillin- resistant *Staphylococcus aureus* (MRSA) has gained global attention as a human pathogen in hospital and in communities. Recent report of MRSA infection and colonization of dogs and cats indicate that MRSA has apparently emerged as a pathogen of animals as well (Fluit *et al.,* 2006). Most reported MRSA infections in dogs have involved wound and post-operative infections (Pear *et al*., 2007). Most pets are probably infected or colonized as a result of contact with contaminated environment or affected people. However, once infected or colonized, pets can pass MRSA to other pets or to people (Weese *et al.,* 2006). Infections with methicillin-resistance *Staphylococcus aureus* may be more difficult to treat and predispose to increase morbidity and mortality in affected veterinary patients (Frank *et al.*, 2009). Over 75% infectious pathogens are zoonotic in nature. Methicillin-resistant and methicillin susceptible *Staphylococcus aureus* (MRSA and MSSA) has been reported in humans and animals in some parts of the world. This study was designed to investigate the presence of methicillin-resistant and methicillin susceptible *Staphylococcus aureus* among dog infections around Jos and its environs and role of DNase production by *Staphylococcus aureus* isolates in relation to Methicillin-resistance and susceptibility.

MATERIALS AND METHODS

Study area

The study area comprised of three Veterinary clinics located in National Veterinary Research Institute Clinic, Vom, Evangelical Church of West Africa (ECWA) Veterinary Clinic, Bukuru and Plateau State Veterinary Hospital, Jos.

Study population

The study population comprised of 50 dogs from each of the different breeds (local breeds, [Mongrel], German Shepherds [Alsatian], Russian Shepherds [Caucasian] and Rottweiler) attending the three hospitals and clinics. A total of 200 samples were collected.

Sample collection and processing

Ear and cutaneous wound swabs were collected randomly from 200 dogs that were categorized according to age as young (≤ 2 years), middle (>2 – 8 years), or old (> 8 years). Their sex and breed were recorded. Samples were collected generally from both healthy and infected dogs attending the three veterinary hospitals, 60 samples were collected from NVRI Clinic Vom, 80 Samples from ECWA Clinic Bukuru and 60 samples from Plateau State Veterinary Hospital Jos. The duration of sample collection lasted for one month. The samples were collected using sterile swab sticks in batches and transported immediately to the laboratory for processing.

Media

The Media used in the study included Brain heart infusion broth, Nutrient agar, Blood agar, Oxacillin agar and DNAse agar. These media were prepared, checked for purity and then kept in the refrigerator prior to use.

Isolation and identification

Each swab sample was inoculated into brain heart infusion broth and incubated at 37°C for 24 hours. After 24 hours, each broth culture sample was aseptically applied to a small area (the pool) of the nutrient plates plus 7% NaCl and blood agar plate whose surface has been dried in the incubator shelf at 37°C for 10 minutes prior to use. Each inoculum was aseptically streaked out from the well to obtain discrete colonies. The plates were then incubated aerobically at 37°C for 24 hours. The characteristics golden yellow colonies were aseptically sub-cultured onto another7% NaCl and blood agar and identified using standard microbiological methods based on morphological, physiological and biochemical characteristics according to Barrow *et al.,* (1993; Cheesbrough, 2004). Isolates based on morphological, physiological and biochemical characteristics that were considered as *Staphylococcus aureus* and were further characterized for MRSA and MSSA by DNase production.

DNase Agar Test

Using a sterile wireloop or swab, the isolate was spot inoculated or streaked across the plate. Each test area was labeled clearly and incubated at 37°C overnight. The plate was flooded with 1ml/L hydrochloric acid solution and excess acid was tipped off. A clear zone around the colony indicates positive test, no clear zone around the colonies indicate negative test

Resistance and Susceptibility test

Susceptibility or resistance testing on all the isolates which were *Staphylococcus aureus* was done by means of the agar screening method on nutrient agar containing 6mg/ml of oxacillin (Coxacillin, 500mg**,** Hovid, Malaysia) and 4% sodium chloride. The *Staphylococcu aureus* isolates were standardized to 0.5Mcfarland standard. The standardized suspensions were spot inoculated aseptically onto nutrient agar plates. The plates were incubated for 24 hours at 37°C (NCCLS, 2000). The diameter of the zone of inhibition produced by each antibiotic disc was measured, recorded and all isolates were classified as resistant, intermediate and susceptible based on the standard interpretative chart according to the National Committee for Clinical Laboratory Standards (NCCLS) diameter sizes (NCCLS, 2002; Cheesbrough, 2004).

RESULTS

A total of 200 samples collected from ear and cutenous wounds were screened for *Staphylococcus aureus*, a total of 135 (67.5%) of the isolates were found to be *Staphylococcus aureus* based on morphological, physiological and biochemical characteristics. Table 1 shows the number of samples collected from 3 different veterinary hospitals and the *S. aureus* infected and non infected cases. Table 2 shows the distributions of samples collected based on their age, sex and breed. Table 3 shows the number of samples collected from ear and cutenous wound swabs. Table 4 shows the resistance and susceptibility of screening of *Staphylococcus aureus* isolates. The prevalence of MRSA and MSSA among the *Staphylococcus aureus* is shown in Table 5.

Table1: Distribution of *Staphylococcus aureus* infections in Dogs based on different locations

Hospital	No of samples collected	Infected cases	Percentage (%)	Uninfected cases	Percentage (%)
NVRI Clinic, Vom	60	40	20	20	10
ECWA Clinic, Bukuru	80	60	30	20	10
Jos, Plateau state	60	35	17.5	25	12.5
Total	200	135	67.5	65	32.5

Table 2: Age, Sex and Breed distribution of *Staphylococcus aureus* infections in Dogs

Parameters		No of samples collected	Infected cases	Percentage (%)	Uninfected cases	Percentage (%)
Age (Years)	≤ 2	60	30	15	30	15
	> 2–8	60	45	22.5	15	7.5
	> 8	80	60	30	20	10
Total		200	135	67.5	65	32.5
Sex	Male	100	66	33	34	17
	Female	100	69	34.5	31	15.5
Total		200	135	67.5	65	32.5
Breed	Mongrel	50	37	18.5	13	6.5
	Alsatian	50	35	17.5	15	7.5
	Caucasian	50	32	16	18	9.0
	Rottweiler	50	31	15.5	19	9.5
Total		200	135	67.5	65	32.5

Table 3: Distribution of *Staphylococcus aureus* infections based on ear and cutaneous wound in Dogs

Source	No of Samples Collected	Infected Cases	Percentage (%)	Uninfected Cases	Percentage (%)
Ear	100	60	30	40	20
Cutaneous wound	100	75	37.5	25	12.5
Total	200	135	67.5	65	35.5

Table 4: Distribution of Oxacillin resistance and susceptible *Staphylococcus aureus* isolates in Dogs

Source	Number sampled	Resistance		Susceptibility	
		Number	Percentage (%)	Number	Percentage (%)
Ear	60	10	7.41	50	37.03
Cutaneous wound	75	26	19.26	49	36.30
Total	135	36	26.6	99	73.33

Table 5: Distribution of Methicillin Resistance Methicillin Susceptible *Staphylococcus aureusisolates in Dogs*

Source/ Number Sampled	MRSA		MSSA	
	Number	Percentage (%)	Number	Percentage (%)
Ear; 60	10	7.41	50	37.03
Cutenous wound; 75	26	19.26	49	36.30
Total 135	36	26.67	99	73.33

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been proven to be one of the most world wide spread nosocomal and community pathogen of the 20th century (Nimmo *et al.*, 2000) and its increasingly developing resistance to many antibiotics (Lowy, 2003). An overall occurrence rate of 135 (67.5%) of *Staphylococcus aureus* was obtained from infected dogs in the study. The colonization rate of *Staphylococcus aureus* in this study were 66 (33%) and 69 (34.5%) for male and female dogs respectively (Table 2). This finding is in agreement with the report of Nester *et al.*, 2001, that 20% of healthy individuals have continually positive *Staphylococcus aureus* infections for a year or more while over 60% will be colonized at sometime during a given year. The frequency of the cutaneous wounds is markedly higher compared to the ear swabs under the infected cases 37.5% against 30% respectively (Table 3). The same was observed concerning their resistance to methicillin which gave19.26% against 7.41% (Table 4) respectively. This may be because of the higher exposure of the cutaneous wounds to the environment which can contribute to the transmission, adhesion and proliferation of the organism in the wound. The various breeds, sex, and age of the dogs diagnosed have narrow difference as observed in (Table 2). This implies that the pathogen has no special affinity for any of those parameters mentioned. In this study, the organisms were cultured into blood agar and nutrient agar plus 7% sodium chloride respectively. This study has shown the existence and occurrence rate of MRSA 36 (26.67%) and MSSA 99 (73.33%). The percentage ressistance to methicillin (26.67%) is higher compared to 8.9% recorded in Loeffler *et al.*, (2005) which was a prevalence study of MRSA among dogs, cats and veterinary hospital staff. The occurrence rate of multi-drug resistance should be of great and immerse concern to the health professionals and all members of the society because transmmission of infections caused by these strains is readily established by close contact (Xander *et al.*, 2006).

Conclusion

The study has established the existence of Methicillin-resistant and susceptible *Staphylococcus aureus* infections in dogs, in Jos and its environs. The MRSA isolated showed multiple drug resistance to beta-lactams commonly prescribed antibiotics. The society is presently characterized with inappropriate prescription, unethical dispensing and

indiscriminate use of antibiotics. Antimicrobial drug use in animals may increase the likelihood of selection for multi-drug resistant bacteria such as MRSA. This increases the rate at which most antibiotics are losing potency in the treatment of infections. Antibiotics are also sometimes prescribed without determining bacterial sensitivity. All these encourage the emergence of resistant strains. It could be concluded therefore, that there is an urgent need to re-assess policies on antibiotic use within and outside the hospital environment. Therefore, control of multiple drug resistance will provide a major challenge to both the health care and the society in general. In order to contain the spread of MRSA, there is need for the diagnostic and research laboratories to screen properly to give early warning(s) of the presence of resistant organisms. This will allow the assessment of barrier and infection control techniques. The use of antibiotics, in livestock and animal feeds need to be adequately controlled. Uncontrolled antibiotics usage may aid the transmission of resistant strains from animals or livestock to humans. Production of newer antibiotics may also be required to meet the challenge of treating patients with drug resistant MRSA infections.

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