
Studies Of Methicillin-Resistant And Susceptible Staphylococcus Aureus Isolates From Dogs Infection

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Abstract

Methicillin-resistant staphylococcus aureus (MRSA) has become a pathogen of animals and there is concern over transmission of Methicillin-resistant staphylococcus aureus (MRSA) between animals and humans. Various hospitals based studies have described the incident of MRSA and carriage of the organism in health care worker. MRSA infections in animal are associated with exposure to medical hospitals extensive wounds, prolonged hospitalization and immunosuppression. The study investigated the existence of methicillin resistant and susceptible staphylococcus aureus isolates from dogs, around Jos and its environs and its public health implications. Standard procedures were employed for isolation, screening and susceptibility testing. A total of 135 staphylococcus aureus were isolated, 36 samples were methicillin-resistant and 99 samples were susceptible to the antibiotics.

Keywords: Staphylococcus aureus, Methicillin, Infection, hospitals, MRSA

Introduction

The advent of antibiotics during the 1940s and 1950s gave clinicians weapon against Infections that one wiped out the entire populations. The discovery and subsequent development of these antimicrobial agents revolutionized medical care worldwide. Therefore, mankind has been so pleased with the ability to conquer diseases. However, the dawn of the antibiotic era was

quickly accompanied by the development of the numerous problems including the emergency 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).

Consequently, diseases caused by these pathogens, for example *staphylococcus aureus* are becoming increasingly more difficult to treat as a causative organism, accumulate new antimicrobial resistance determinants (Fraimow & Abrutyn, 1995). *Staphylococcus aureus* is a pathogen of greater concern because of its virulence (Boyle-Vavra & Daum, 2007).

The organism has the ability to cause a wide range of infections, from minor skin and soft tissues infection to life threatening pneumonia and toxicosis, all due to its capacity to adapt to different environmental conditions (Lowy, 1998; Lowy, 2003).

Staphylococcus aureus is recognized as one of the most important bacteria of human and veterinary hospital environment all over the world (Lina, *et al.*, 1999). *Staphylococcus aureus* is a common skin and nasopharynx 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).

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 (Boyle-Vavra & Daum, 2007). The beta-lactamase enzyme destroys the penicillin antibiotic by hydrolyzing the beta-lactam ring and this decreases the usefulness of the penicillin antibiotics (Boyle-Vavra & Daum, 2007).

Methicillin a beta-lactamase insensitive beta-lactam, provided new treatment options for PRSA infections in the late 1950s, but methicillin-resistant *staphylococcus aureus* (MRSA) that are cross resistant to all beta-lactams soon emerged, primarily in health care environment (Boyle-Vavra & Daum, 2007).

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, *et al.*, 2004).

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Methicillin resistant *staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult –to- treat infections in human and animal. It may also be called multi drug resistant *staphylococcus aureus* or oxacillin –resistant *staphylococcus aureus* (ORSA),(Klevens, *et al.*, 2007).MRSA is by definition, any strain of *staphylococcus aureus* bacteria that have developed resistance to betalactam antibiotics which include the penicillins (methicillin, Dicloxacillin, Nafcillin, oxacillin etc.) and the cephalosporins (Moreno,*et al.*, 2007) Methicillin- resistant *staphylococcus aureus* was discovered in 1961 in the united kingdom. It made it first major appearance in united states in 1881 among intravenous drug users (carmeli *et al.*, 2005). During the past 2 decades, methicillin –resistant *staphylococcus aureus* (MRSA) has gained global attention as a human pathogen in hospital and incommunities. Recent report of MRSA infection and colonization of dogs have involved wound and post-operative infections (Weese & Lefebvre 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). Infection with methicillin – resistance *staphylococcus aureus* may be more difficult to treat and predispose to increase morbidity and mortality in affected veterinary patients (Van Duijkeren,*et al.*, 2004)

Objectives Of the Study

The objectives of this research include:

- i. To ascertain the existence of methicillin –resistant and susceptible *staphylococcus aureus* infections in dogs and its environs its public health implications.
- ii. To determine the occurrence of MRSA and MSSA *staphylococcus aureus* among the dog breeds.
- iii. To determine the occurrence based on age and sex of the breeds.
- iv. To determine the role of Dnase production by *staphylococcus aureus* isolates in relation to methicillin- resistance and susceptibility
- v. To recommend the way forward and control measures based on the study finding.

Research Methodology

Population of the Study

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

Media

The media used in the study included Brain heart broth, Nutrient agar, Blood agar, oxacillin agar, and DNase agar. These media were prepared and refrigerated prior to use.

Sample Collection

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). Sex whether male or female and breed. Samples were collected generally from both healthy and infected dogs attending the three veterinary hospitals, 60 samples were collected from NVRI clinic Vom, 80Samples from ECWA clinic Bukuru and 60 samples from plateau State veterinary hospital Jos. The duration of sample collection lasted for 4 weeks (14thJune -12thJuly 2010). The samples were collected using a sterile swab sticks in

batches and transported immediately to the laboratory for processing.

Cultural Procedure (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 well) of the nutrient plates plus 7% Nacl and blood agar plate whose surface has been dried in the incubator shelf at 37°C for ten 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 isolated and subcultured using established microbiological methods that include colonial morphology, Gram stain characteristics and biochemical test (Cheesbrough, 2004). Isolates that were Gram –positive cocci in clusters, catalase and coagulase positive were considered as *staphylococcus aureus*, and were further characterized for DNase production

DNase Agar Test Screen Given By Nccls,2000.

Using a sterile wire loop or swab, spot inoculate the isolate or streak across the plate. Each test area is labeled clearly.

Incubate the plate at 37°C overnight. Flood the plate with 1m/L hydrochloric acid solution. Tip off the excess acid. A clear zone around the colony indicates positive test, no clear zone around the colonies indicate negative test (Barrow & Feltham, 1993; Cheesbrough, 2004).

Resistance and susceptibility screening using the Oxacilin Agar screen given by Nccls, 2000.

Susceptibility testing on all the isolates which were *staphylococcus aureus* was done by means of the agar screening method on nutrient agar containing 6Ng/mL of oxacillin (cloxacilin, 500mg, Hovid, Malaysia) and 4% sodium chloride. The *staphylococcus aureus* isolates were standardized to 0.5 McFarland standard. The standardized suspensions were spot inoculated aseptically onto the nutrient agar plates. The plates were incubated for exactly 24 hours at 30° (Kiehlbauch *et al.*, NCCLS, 2000).

Interpretation Of Result

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, which fulfill the World Health Organization and performance standard for antimicrobial disc susceptibility testing were recorded (Kiehlbauch *et al.*, NCCLS, 2002; Cheesbrough, 2004).

Discussion

Methicillin-resistant *staphylococcus aureus* (MRSA) has been proven to be one of the most world wide spread nosocomial and community pathogen of the 21st century (Huijsdens, *et al.*, 2000; Cosgrove, *et al.*, 2003) and it's increasingly developing resistance to many antibiotics (Lowry, 2003). An overall occurrence rate of 135 (67.5%) of *staphylococcus aureus* was obtained from infected dogs in this study were 66 (66%) and 69 (69%) for male and female dogs respectively. This finding is in agreement with the report of Nester *et al.*, 2001, that 20% of healthy individual continually have positive *staphylococcus aureus* infections for a year or more while over 60 % will be colonized at some time during a given year.

For any bacterium to be propagated for any purpose, it is necessary to produce the appropriate culture medium and depending on the state of the organism at a particular point will equally affect the growth of the organism. In this study, the organisms were cultured into blood agar

and nutrient agar plus 7% sodium chloride respectively.(Cosgrove,*et al.*, 2003) The number of growth on blood agar was more than the one on nutrient agar plate. This study has shown the existence and occurrence rate of MRSA 36 (26.6%) and MSSA 99 (73.3%).

The occurrence rate of multi-drug resistant should be of great and immense concern to the health professionals and all members of the society because transmission of infections caused by these strains is readily established by close contact (Huijsdens,*et al.*,2006).

Conclusion

The study has established the existence of methicillin –resistant and susceptible *staphylococcus aureus* infections in dogs, around jos and its environs. The MRSA isolated showed multiple drug resistance to beta-lactams commonly prescribed antibiotic.

The society is presently characterized with inappropriate prescription, unethical dispensing and indiscriminate use of antibiotics. Antimicrobial drug use in animal would increase the likelihood of selection for multi-drug resistant bacteria such as MRSA. This increase the rate at which most antibiotics are losing the battle in the treatment of infections. Antibiotics are also sometimes prescribed without

determining bacteria sensitivity to antibiotics. All these encourage the emergence of resistant strain. It could be concluded therefore, there is an urgent need to reassess policies on antibiotic use within and outside the hospital environment. Therefore, control of multiple drug resistance will provide a major challenge to both the healthcare and the society in general.

Recommendation

The following recommendations are essential in the containment of resistance to antimicrobial agents:

1. Formulation and implementation of a national drug policy by the government to ensure rational use of antibiotics.
2. Effective education and training of the public about the appropriateness and limitation of antibiotics to ensure that they are utilized wisely and also the need to adopt the high personal hygiene.
3. Proper prescription of antibiotics and implementation of an agreed control infection policies.
4. Laboratories should screen properly to give early warning of the presence of resistant organisms and allow the

assessment of barrier and infection control techniques.

5. The use of antibiotics, in livestock and animals` feed should be adequately controlled. This may aid the transmission of resistant strain from animals or livestock to humans.

6. Production of newer antibiotics may be required to meet the challenging of treating patients with drug resistant MRSA infections.

Dealing with pets infected with MRSA.

- Avoid contact with the infected area unless cleaning or changing bandages for your pet.
- Wear gloves when cleaning bandages
- Wash your hands thoroughly after contact with your pet.
- Follow your veterinarians instructions carefully in dealing with these MRSA infections and always finish any antibiotic prescribed for your pets

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