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Phenotypic Isolation and Antibiotic Sensitivity to Methicillin-Resistant Staphylococcus Aureus (MRSA) in Horses in Maiduguri and its Environs

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Abstract

This study was conducted to determine Methicillin-resistant Staphylococcus aureus (MRSA) in Horses in Maiduguri and its Environs. A total of 150 nasal swabs were collected from Horses for laboratory examination, the presence of Methicillin-resistant staphylococcus aureus in the horse nasal flora was investigated, the antibiotic susceptibility of Staphylococcus aureus isolate was carried out and MRSA amongst staphylococcus aureus isolates was determine using Oxacillin Resistance Screening Agar Base (ORSAB).

Among the One hunded and fifty (150) nasal swabs collected None hundred and forty one (141) were positive for staphylococcus aureus, while Nine (9) do not show the presence of *staphylococcus aureus* in the nasal flora on Mannitol Salt Agar (MSA). The antibiotic susceptibility test result showed thirty nine (39) isolates were susceptible on Pefloxacin, Gentamycin, Ciprofloxacin, Septrin, Streptomycin antibiotics; thirty nine (39) were resistant on Ampiclox, Amoxicillin antibiotics; twenty four (24) intermediate and fifteen (15) resistant on Rocephin; nine intermediate and twelve (12) susceptible Erythromycin; thirty six (36) resistant and three (3) susceptible on Zinnacef, were performed by disc diffusion method. The result from this study however shows isolate was more susceptible to fluoroquinolones and all were positive on ORSAB, which were mostly identified in the specimen from the apparently healthy horses in Maiduguri and its Environs.

Keyword: Methicillin-Resistant Staphylococcus Aureus (MRSA); Antibiotic susceptibility; Oxacillin Resistance Screening Agar Base (ORSAB); Apparently healthy

Introduction

Staphylococcus aureus (S. aureus) is one of the species of the genus staphylococcus. The organism was first discovered in the United Kingdom in 1961 (Fayomi et al., [1]. It is a gram-positive, non-motile, catalase-positive, coagulase-positive, facultative anaerobic bacterium which belongs to the commensal flora of humans and animal species (Vanderhaeghen et al., [2]. Staphylococcus aureus (S. aureus) is normally found on the skin or in the nares of about 30% of healthy individuals. When (S. aureus) is present without causing symptoms it is called an infection (Fact sheet Review 2007). Multiple body sites can be colonized but the anterior nares are the most frequently colonized sites Wertheim et al. [3]. For humans, this organism is an important cause of foodborne intoxication, pneumonia, post-operative wound infections, and nosocomial bacteremia. Staphylococcus aureus is considered the most resistant of all non-spore forming pathogens, with well-developed capacities to withstand high salt concentrations (7.5-10%) (Talaro and Talaro [4].

Staphylococcus aureus is known to be notorious in the acquisition of resistance to new drugs and continues to defy control measures. Many strains of S. aureus that carry a wide variety of multi-drug resistant genes on their plasmids are known as Methicillin-Resistant Staphylococcus Aureus (MRSA). Methicillin-resistant S. aureus is isolates of S. aureus which have acquired genes encoding antibiotic resistance to all penicillins including methicillin.

The emergence of multiresistant staphylococci and particularly Meticillin-Resistant Staphylococcus Aureus (MRSA) has focused attention on the need for a better understanding of the epidemiology and pathogenesis of staphylococcal disease and the development of more effective methods for their treatment and control. A recent feature of MRSA emergence has been the recognition of the farm animaladapted MRSA Sequence Type (ST) 398, which is now widespread in many countries, particularly in pig farms. Spread of ST398 into human communities and hospitals with consequences for public health is causing increasing concern amongst healthcare providers.

Resistant bacteria arising either in humans, animals, or the environment may spread from one to the other, and from one country to another. I shall conduct deep research in this Antimicrobial resistance diversity using Staphylococcus aureus in Horses as a study model, and go on numerous visits to Veterinary farms, Hospitals and Clinics in Borno state Nigeria to collect samples from Horses for and investigate the resistant S. aureus.

The significance and prevalence of MRSA from colonization and infections in horses in Maiduguri has not been assessed in more detail so far. Therefore, the objectives of the study are to: determine the presence of staphylococcus aureus in the horse nasal flora, determine the antibiotic susceptibility of Staphylococcus aureus isolates, and determine MRSA amongst staphylococcus aureus isolates using ORSAB Figure 1.

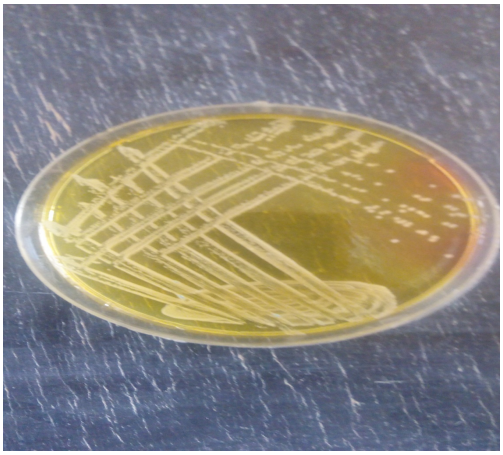

Figure 1: Photomicrograph of Staphylococcus aureus on Manitol Salt Agar (MSA).

Theis reseach project will contribute to the knowledge required for the development, implementation, and maintenance of safe usage of antibiotics in treating Staphylococcus aureus infection in Horses, and contribute to disease surveillance programmes and control of zoonoses in Nigeria.

Material and Method

Study area

The study was conducted in Maiduguri, Borno State which lies between latitude 10.20N and 13.40N longitude 9.80E and 14.40N with an area of 69,436 Sq Km located in the Northeastern corner of Nigeria sharing borders with Niger to the North, Chad to the Northeast and Cameroon to the east. The State has Sahel vegetation in the North and a Sudan Savanna in the South.

Sampling Technique

A convenient sampling technique was employed in the study. A total of 50 Nasal swabs samples were collected from various horses within the study area. Information on each of the sampled horses was collected such as age, sex, and date Figure 2.

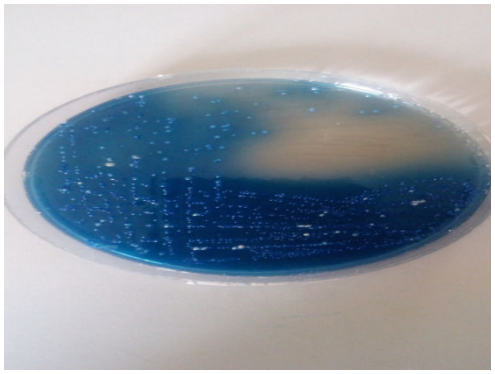

Figure 2: Photomicrograph of Staphylococcus aureus on Oxacillin Resistance Screening Agar Base (ORSAB).

Sample collection procedure

After putting on clean gloves, a sterile swab stick was introduced into the nostril of the Sampled Animals and rolled against the nasal mucosa and then replaced into the swab stick case. The swabs were aseptically transported in an ice pack container to the Department of Veterinary Medicine Laboratory and analysis was done according to Cheesbrough, .

Materials used include

Hand gloves, Culture media (Mannitol salt agar, Nutrient agar, ORSAB), Antibiotic sensitivity discs (Maxi disc high profile +ve), distilled water (Juhel ®), Pasteur loop, cotton wool, sterile swab sticks (Tyconpacey ®), normal saline (Juhel ®), syringes and needles (Agar-Jec ®), permanent marker (Laries ®), aluminum foil, masking tape, test tubes, test tube racks, Bijoux bottles, disposable Petri dishes, conical flasks, measuring cylinder, disposable pipettes (Cosar ®), micropipette (Per-Fect ®), refrigerator, incubator (Gallenhamp ®), weighing balanc \e (Gallenhamp ®), Vortex machine (Gallenhamp ®), centrifuge (Gallenhamp ®), hot air oven (Gallenhamp ®), autoclave (Gallenhamp ®), Bunsen burner, mini-columns.

Media

The following media Mannitol salt agar (CM2), Nutrient agar (CM3) and ORSAB (Oxacillin Resistance Screening Agar Base) were prepared and used according to the Manufacturer's instructions.

Antibiotic impregnated discs and their concentrations

All antibiotic discs were obtained from Oxoid (Basingstoke, UK). These antimicrobial agents were selected based on the class differences in their modes of action and mechanisms of resistance. The details of the antibiotics used and their concentrations are given in a table in the appendix.

Bacterial isolation and identification

Swabs from both horses were analyzed for the presence of S. aureus as described by Cheesbrough (2002). Isolation of S. aureus was made by culturing the sample on Mannitol salt agar prepared according to the conventional technique. The cultured plate was incubated at 370C aerobically for 24 hours and thereafter examined for the presence of Staphylococcus like colonies. The suspected Staphylococcus colonies (yellowish colonies from Mannitol fermentation) were selected and subcultured on to Mannitol salt agar.Colonies typical of Staphylococcus were further subcultured onto the same media plates from which subsequent growth was examined using biochemical test; catalase and coagulase test positive for the presence of Staphylococcus aureus. Cheebrough [5].

Biochemical Test

Catalase test

A drop of diluted 3% Hydrogen peroxide was put on a clean-grease free slide and a colony of test organisms was picked using a sterile wire

loop and mixed in the diluted Hydrogen peroxide and was examined for gas bubble which indicates catalase-positive reaction. The test was used to differentiate Staphylococcus species (Microbiology A laboratory manual).

Coagulase test

A drop of rabbit plasma was put into a clean glass slide, and a colony of Staphylococcus was picked using a sterile wire loop added and mixed. A positive coagulase test showed clumping immediately. This was used to differentiate pathogenic Staphylococcus aureus from non-pathogenic staphylococci laboratory exercises in microbiology).

Oxacillin Resistance Screening Agar Base (ORSAB)

Oxacillin Resistance Screening Agar Base (ORSAB) is used for the screening and isolation of methicillin-resistant S. aureus from clinical samples. The medium was allowed to reach room temperature, a colony of Staphylococcus was picked using a sterile wire loop and inoculated over a small area near the edge of the plate containing the media, and then four-quadrant streak was performed. The plates were incubated aerobically at 350C and examine after 24 hours for typical MRSA colonies. MRSA colonies appeared as intense blue colonies on the agar surface, the growth of other bacteria was inhibited but those able to grow on the media were typical colonies Figure 3.

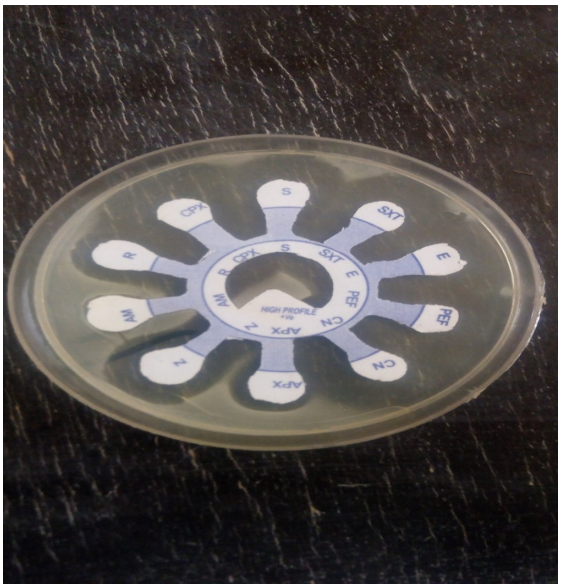

Figure 4: Photomicrograph of Staphylococcus aureus on nutrient agar with impregnated antibiotic disc.

Antibiotic Sensitivity Test

All identified S. aureus spp were examined for antimicrobial susceptibility to determine methicillin resistance by disc diffusion method using Nutrient agar according to the clinical and laboratory standard institute guidelines (CLSI, 2010).

The 10 tipped multiple susceptibility discs were used and made up of the following antimicrobial impregnated discs.

- Pefloxacin (PEF) 10 ug
- Gentamycin (CN) 10 ug
- Ampiclox (APX) 30 ug
- Zinnacef (Z) 20 ug
- Amoxacillin (AM) 30 ug
- Rocephin (R) 25 ug
- Ciprofloxacin (CPX) 10 ug
- Septrin (SXT) 30 ug
- Streptomycin (S) 30 ug
- Erythromycin (E) 10 ug

Results

Among the One hunded and fifty (150) nasal swabs collected None hundred and forty one (141) were positive for staphylococcus aureus, while Nine (9) do not show the presence of staphylococcus aureus in the nasal flora on Mannitol salt agar (MSA).

The antibiotic susceptibility test result showed thirty nine (39) isolates were susceptible on Pefloxacin, Gentamycin, Ciprofloxacin, Septrin, Streptomycin antibiotics; thirty nine (39) were resistant on Ampiclox, Amoxicillin antibiotics; twenty four (24) intermediate and fifteen (15) resistant on Rocephin; nine intermediate and twelve (12) susceptible Erythromycin; thirty six (36) resistant and three (3) susceptible on Zinnacef, were performed by disc diffusion method.

The result from this study however shows isolate was more susceptible to fluoroquinolones and all were positive on ORSAB, which were mostly identified in the specimen from the apparently healthy horses in Maiduguri and its Environs Tables 1-4.

Test	Horse No. tested	No. (%) Positive
Manitol Salt Agar (MSA)	150	141 (94.0)
Catalase	141	129 (91.5)
Coagulase	141	126 (89.4)
ORSAB	114	39 (34.2)

Table 1: Morphological and Biochemical Characteristics of Staphylococcus aureus (S. aureus) and Methicillin Resistant Staphylococcus Aureus (MRSA) in Horses in Maiduguri and its Environs.

No. tested	Staphylococci isolates No. (%)+Ve	S. aureus No. (%) +Ve	MRSA No. (%) +Ve
150	141 (94.0)	114 (80.6)	39 (34.2)

Table 2: Prevalence of methicillin resistant staphylococcus aureus.

S.N	Antibiotis	Susceptible	Intermediate	Resistance
1	Pefloxacin	39	0	0
2	Gentamycin	39	0	0
3	Ampiclox	0	0	39
4	Zinnacef	3	0	36
5	Amoxicillin	0	0	39
6	Rocephin	0	24	15
7	Ciprofloxacin	39	0	0
8	Septrin	39	0	0
9	Streptomycin	39	0	0
10	Erythromycin	12	27	0

Table 3: Antibiotic sensitivity test of the MRSA isolates of the samples.

Antibiotics	Concentration	Resistance	Intermediate	Susceptibility
Oxacillin	5 µg	≤10 mm	11-12 mm	≥ 13 mm
Penicillin G	10 units	≤28 mm	-	≥ 29 mm
Gentamicin	10 µg	≤12 mm	15-16 mm	≥ 15 mm
Erythromycin	5 µg	≤ 13 mm	14-22 mm	≥ 23 mm
Sulphamethoxazole	25 µg	≤ 12 mm	13-16 mm	≥ 17 mm
+ Trimethoprim				
Tetracycline	30 µg	≤ 14 mm	15-18 mm	≥ 19 mm
Ciprofloxacin	5 µg	≤ 15 mm	16-20 mm	≥ 21 mm
Chloramphenicol	30 µg	≤12 mm	13-17 mm	≥18 mm
Cefoxitin	30 µg	≤24 mm	-	≥25 mm
Clindamycin	5 µg	≤10 mm	11-12 mm	≥13 mm
Cephazolin	30 µg	≤10 mm	11-12 mm	≥13 mm

Table 4: Antibiotics disc used and their break point for testing S. aureus isolates in maiduguri and its enivirons.

Discussion

This study was conducted to determine the phenotypic isolation of Methicillin Resistance Staphylococcus Aureus (MRSA) in horses in Maiduguri and its Environs. The phenotypic results in this study showed out of the 150 horses sampled 141 (94.0%) were positive which appeared higher compared to 9.5% and 3.0% reported by Rahimi [6] in cattle in Maiduguri Metropolitan.

The difference might be due to differences in the type of study because this was done in equine.

Out of 114 (80.6) S. aureus isolated in this study, 39 (34.2) were resistant to oxacillin which disagreed with the result of John Hwa Lee [7] which shows 15 (3.6%) out of 421 S. aureus isolates were positive, isolated from Major Food animals. feces, milk, feed material, joint, trachea, uterus, and meat specimens of beef cattle, dairy cattle, pigs, and chickens in Korea. The differences might be due to the type and method of sample collection, season of sampling, sample size, and the animal species used [8].

The result of antibiotic susceptibility from this study revealed that all MRSA isolates were resistant to Ampiclox, Amoxicillin and Zinnacef antibiotics, while susceptible to Pefloxacin, Gentamycin, Ciprofloxacin, Septrin, Streptomycin antibiotics and intermediate to Rocephin, Erythromycin, which agreed with the result of John Hwa Lee, 2003, who reported all MRSA isolates were resistant to penicillin and ampicillin and were less susceptible to erythromycin, gentamicin, and kanamycin, and More susceptible to trimethoprim-Sulfamethoxazole and fluoroquinolones, such as ciprofloxacin, ofloxacin, and norfloxacin, from food animals in Korea.

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Conflict of Interest:

Babangida Abdullahi, Muhammad maaruf Ibrahim, Esther Mshelia and AminaLawan Adam as the Authors of the above named research project declare that there is no conflict of interest in this study.

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