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Research Paper

Prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) in Fattening Lots in Maiduguri, Borno state, Nigeria

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ABSTRACT: *The study was aimed at determining the presence of MRSA in cattle in fattening lots in Maiduguri phenotypically. Examination of 150 nasal swab samples from the nostril of apparently healthy cattle reveal 78 (32%), 62 (42.6%) and 32 (21.3%) were positive for Staphylococcus, S. aureus and MRSA respectively. The identify age as a risk factor for MRSA in cattle in fattening lots in the study area ($p<0.05$; OR: 3.226; CI: 1.295 to 8.037). The result of antimicrobial susceptibility revealed that the MRSA isolates in this study were highly resistance to Oxacillin (100%), Penicillin (100%), Tetracycline (100%), Cefoxitin (100%), Cephazolin (100%) and Sulphamethoxazole+Trimethoprin (86%). The isolates were also found to susceptible to Ciproflaxacin (100%) and Gentamycin (87.5%). The study showed that ciprofloxacin may be the drug of choice in the treatment of MRSA in the study area. The overall 100% of the MRSA isolates were multidrug resistant (MDR).*

Keywords: *Methicillin-Resistant Staphylococcus aureus (MRSA), Multi-drugs resistant, Antibiogram, infectious disease.*

I. INTRODUCTION

There is increasing global concern for the spread of antibiotic resistant bacteria particularly multi-drug resistant zoonotic pathogens [1, 2, 3]. Associated with this concern is the mutual assertion that both medical and veterinary use of antimicrobial agents in promoting the emergence and rise in the prevalence of these resistant pathogens. Many researchers have investigated the role of animal food products such as meat [4] and milk [5, 6, 7] as sources of resistant zoonotic bacteria. One of such bacteria is Methicillin-Resistant *Staphylococcus aureus.* Methicillin-resistant Staphylococcus aureus (MRSA) are the strains of *S. aureus* that are resistant to all available penicillins and other β-lactam antimicrobial drugs [8, 9]. This resistance is caused by an alternative penicillin-binding protein, called PBP2a. PBP2a is encoded by the *mec*A gene located in the mobile genetic element called Staphylococcal cassette chromosome (SCC*mec*) [10].

Traditionally MRSA has been considered a hospital-associated pathogen (HA-MRSA) [11]. Prolong hospital stay, intensive–care unit, prolong antibiotic treatment, surgical intervention and/or close contact with infected or colonized MRSA-positive individual are risk factors for attracting HA-MRSA [12]. Until the 1990s, infections with MRSA were rarely observed in extramoral communities [11]. However since the mid 1990s, MRSA strains were increasingly documented in healthy people without healthcare associated risk factors [11]. These cases were referred to as community-associated MRSA (CA-MRSA). Analysis of the genetic background of these CA-MRSA strains has shown a clear distinction from typical HA-MRSA, CA-MRSA and HA-MRSA belong to different sequence types and in addition carry different *SCCmec* type [11].

Recently, MRSA has been found to be emerging in livestock [13]. Animals act as reservoir of MRSA, and the bacterium can be transmitted to humans in close contact with MRSA colonized animals. MRSA from

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these reservoirs has been referred to as Livestock Associated-MRSA (LA-MRSA) to distinguish it from HA-MRSA and CA-MRSA types [14]. The initial case of LA-MRSA in humans was described in 2005 in a 6 month old girl admitted to a hospital for invasive surgery in Netherlands [15].

To the knowledge of the authors, no similar work has been carried in this geographical zone that may highlight the epidemiological characteristic pattern of MRSA isolates. The peculiarity of the study area boarded by three republics involved importation of animals from the three neighboring countries of Chad, Cameroun and Niger and transported to other part of Nigeria, especially after fattening, unregulated sales of antimicrobials agents, all these are known predisposing factors for emergence of resistant strains.

II. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in fattening lots in Maiduguri metropolis from October, 2015 to January, 2016. Maiduguri is the capital and the largest urban Centre of Borno State, North-Eastern Nigeria. The State lies between latitude 110 32' North and 110 40' North and latitude 130 20' East and 130 25'East between the Sudan savannah and Sahel savannah vegetation zones, characterized by short rainy season of 3-4 months (June-September) followed by a prolonged dry season of more than 8 months duration [16].

2.2 Sample Size Estimation

The sample size was determined using Thrustfield formular [17]. Using a previous prevalence of 9.5% [18]. The calculated sample size was 143 samples for more precision 150 nasal samples were collected from cattle from fattening lots.

2.3 Sample Collection

Nasal swabs samples were collected from the nostrils of cattle using commercially available sterile swab sticks (MWE Medical wire, Corsham, Wiltshire, England) within Maiduguri between February to May 2016. The samples were labeled according to sex and age and transported in an ice pack to the Veterinary Microbiology laboratory of the faculty of veterinary medicine, university of Maiduguri for analysis. The cattle breeds included were Amballa, Red Fulani, White Fulani and Kuri fattened under intensive management system. Fifteen fattening lots, one each from the 15 wards of the metropolis were selected for the study, with each having cattle population greater than 20. From each of the selected lot, samples were collected from the nasal mucosa of 10 cattle thereby making the sample size of 150 cattle.

2.4 Isolation of *S. aureus*

Sterile cotton-tipped swabs stick were inserted into the inner nasal septum of the anterior nares of cattle and rubbed several times, removed and capped, label appropriately and immediately transported to the laboratory in an ice pack and analysed bacteriological for the presence of *S. aureus* by culturing the sample on Blood agar (BA) and Mannitol salt agar (MSA) obtained from Oxoid Ltd. Basingstoke, Hamshire England and prepared according to the manufacturers instruction. The cultured plate were incubated at 37^0C aerobically for 24 hours and thereafter examined for the presence of *Staphylococcus* like colonies with haemolysis on blood agar and yellowish appearance on mannitol salt agar. Plates were further analysed using standard procedures: colonial morphology, Gram reaction, catalase, tube coagulase and DNase [19].

2.5 Identification of Methicillin Resistance *Staphylococcus aureus*

Oxacillin Resistance Screening Agar Base is a medium for the screening of Methicillin resistant *Staphylococcus aureus* (MRSA), the medium is nutritious, selective and contains peptones for growth. It has a high salt and lithium chloride concentration to suppress non-staphylococcal growth; with Mannitol and aniline blue, for the detection of Mannitol fermentation. The antibiotics contained in ORSAB Selective Supplement are Oxacillin at 2 mg/liter to inhibit Methicillin sensitive Staphylococcus aureus (MSSA) and Polymyxin B for the suppression of other bacteria that are able to grow at such a high salt concentration. Example Proteus spp. typical colonies of MRSA are intense blue in color on a colorless background enabling the organism to be more easily identified in mixed culture than the pale yellow colonies seen on Mannitol Salt Agar.

2.6 Antibiotic Susceptibility Testing

The Antibiotic Susceptibility Testing (ATS) of MRSA isolates was determined according to the method of Bauer-Kirby [20] by using commercially prepared disc (Oxoid, UK) with known concentration of antibiotics. Freshly sub-cultured MRSA and well isolated colonies from ORSAB plates were emulsified in 3-4 m of sterile normal saline. The turbidity of the suspension was adjusted to the turbidity of standard equivalent to 0.5 McFarland turbidity standards [21, 22]. Muller Hinton Agar Medium was prepared and the standardized overnight culture of each isolate that were constituted to McFarland turbidity standard (containing

approximately 10^6 cfu/ml) was used to flood the surface of Mueller Hinton agar plates and excess was drained off and allowed to dry while the Petri dish lid was in place [22]. Five antimicrobial discs were dispensed into each inoculated plates at equidistant and incubated at 35^{o}C for 24hrs. Zone of inhibition were measured in millimeters (mm) using vernier caliper. The sizes of the zones of inhibition were interpreted according to [21] criteria. The following ten antibiotics were tested; Penicillin G (PEN) 10units, Cefoxitin (FOX) 30ug, Sulphadiazine and Trimetoprine (SXT) 25ug, Ciproflaxacin (CIP) 5ug, Erythromycin (E) 15ug, Cephazoline (KZ) 30ug, Chloramphenicol (C) 30ug, Gentamycin (GN) 10ug, Tetracyclin (TE) 30ug, and Oxacillin (OX) 1gm (Oxoid,UK). For the interpretation of susceptibility towards Oxacillin disc, growth within the zone of inhibition was considered indicative of Methicillin resistance. According to the classification criteria given by CLSI (2014), a diameter of inhibition zones of ≤ 10, 11-12, and ≥ 13 by 1ug of Oxacillin is categorized as resistant (R), intermediate (I) or susceptible (S) to Oxacillin accordingly. For Cefoxitin disc, a diameter of inhibition zones of ≤ 24 and ≥ 25mm correspond to the class of Staphylococci considered as resistance or susceptible for Oxacillin.

2.7 Statistical Analysis

Data generated were analyzed using computer software grashed instat (2000). Variables were assessed for association with MRSA colonization using Chi Square, odds ratio at 95% confidence interval for statistical association. Also tables and percentages were used.

III. RESULTS

Examination of a total of 150 nasal swabs from the nostrils of cattle in fattening lots revealed 78 (52%), 62 (42.6%) and 32 (21.3%) were positive for Staphylococcus, *S. aureus* and MRSA respectively. The prevalence of MRSA from male and female cattle was 29 (19.3%) and 3 (2%) respectively, however there was no statistical association between MRSA and sex of cattle examined at $p>0.05$ (Table 1), this inferred that both sex were equally predisposed to the pathogen and have shared the same risk of carriage in the study area. The prevalence of MRSA from adult and young cattle was 25 (16.7%) and 7 (4.6%) respectively, there was statistical association between MRSA and age of cattle examined ($p<0.05$; OR: 3.226; CI: 1.295 to 8.037) (Table 1). The result of antimicrobial susceptibility test revealed that the isolates of MRSA were highly resistance to Oxacillin (100%), Penicillin (100%), Tetracycline (100%), Cefoxitin (100%), Cephazolin (100%) and Sulphamethoxazole + Trimethoprim (81.2%) whereas they showed 100% and 87.5% susceptibility to Ciproflaxacin and Gentamycin respectively. (Figure 1). Multi drug resistance (MDR) profile of MRSA isolates are shown in (Table 2). This indicates that all the MRSA isolated in this study are Multi drug resistant isolates.

Table 1: Prevalence of MRSA in cattle of different age and sex distribution in fattening lots in Maiduguri, Borno State.

Sex	**No. of Animal Sampled**	**No. *S. aureus* +Ve (%)**	**No. MRSA +Ve (%)**
Male	123	51 (34)	29 (19.3)[a]
Female	27	13 (8.6)	3 (2)[a]
Total	150	64(42.6)	32(21.3)
Age			
Adult (1-2 years)	87	41 (27.3)	25 (16.7)[a]
Young (≥ 2 years)	63	23 (15.3)	7 (4.6)[b]
Total	150	64 (42.6)	32 (21.3)

Values denoted by the same superscripts in the 4th column did not differ significantly (P>0.05): Chi-Square and Odds Ratio (OR). Values denoted by different superscripts in the 4th column differ significantly (P < 0.05): Chi-Square and Odds Ratio (OR).

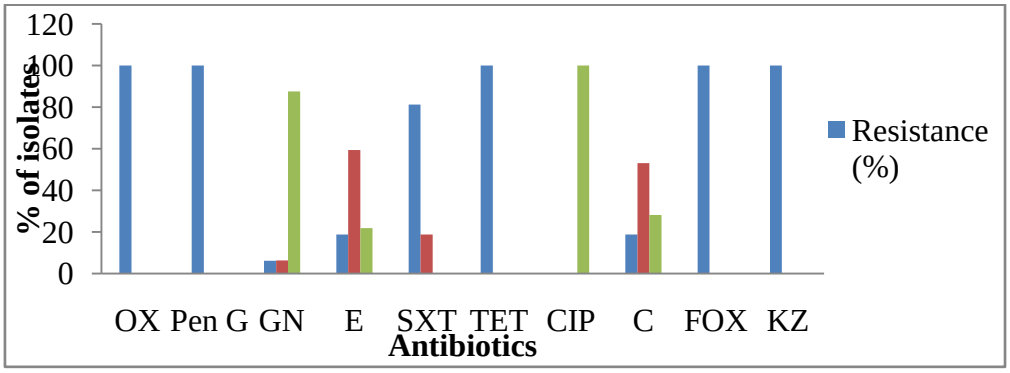

Figure 1: Antibiogram of MRSA Isolates from cattle in fattening lots in Maiduguri, Borno State. OX=Oxacillin, PEN=Penicillin, GN= Gentamycin, E=Erythromycin, SXT=Sulphadiazine and Trimetoprine, TET=Tetracyclin, CIP=Ciproflaxacin,C= Chloramphenicol, FOX=Cefoxitin and KZ=Cephazoline)

Table 2: Multiple Antimicrobial resistance profile of MRSA isolates from cattle in fattening lots in Maiduguri, Borno State.

Antimicrobial Agents	No. of isolates (%)
PEN, OX, TE	32 (100)
PEN, OX, TE, FOX	32 (100)
PEN, OX, TE, FOX, KZ	32 (100)
PEN, OX, TE, FOX, KZ, S+T	(81.2)
PEN, OX, TE, FOX, KZ, S+T, C	6(18.8)
PEN, OX, TE, FOX, KZ, SXT, C, E	6(18.8)
PEN, OX, TE, FOX, KZ, SXT, C, E, GN	2(6.2)

Key: OX=Oxacillin, PEN=Penicillin, GN= Gentamycin, E=Erythromycin, SXT=Sulphadiazine and rimetoprine, TET=Tetracyclin, CIP=Ciproflaxacin,C= Chloramphenicol, FOX=Cefoxitin and KZ=Cephazoline

IV. DISCUSSION

The prevalence of MRSA in this study was found to be 21% in fattening lots in Maiduguri, Borno state. The finding in this study appear higher compared with the finding of [18] who reported prevalence of 9.5% in cattle slaughtered for human consumption in Maiduguri abattoir. MRSA causes significance epidemiologic and therapeutic challenges in both human and animals. The high prevalence of MRSA in this study is of concern considering the fact that MRSA has zoonotic potentials. The high prevalence in this study could be due to the fact that cattle slaughtered in the abattoir are bought from the neighboring villages and rested for a day or 2 before slaughter where they are exposed to less antibiotics while cattle in fattening lots are kept for a longer period (between 60 and 90 days) for fattening and usually treated with different antibiotics from diverse sources with or without prescription, the site and time of sample collections and techniques applied to search for carriage (enrichment protocols i.e. culture method employed for isolation). The study identified age as a risk factor associated with MRSA in cattle in this study (p>0.05; OR: 3.226; CI: 1.295 to 8.037). This agreed with the finding of other researcher who identified older age as a risk factor of MRSA infection [23, 24]. Antimicrobial susceptibility pattern of MRSA isolates was also investigated indicating high resistance against Oxacillin (100%), Penicillin (100%), Tetracycline (100%), Cefoxitin (100%), Cephazolin (100%) and Sulphamethoxazole +Trimethoprin (86%). This therefore implies that Cefoxitin based assays are particularly important for low level Oxacillin resistant MRSA detection (25). Penicillin and Tetracycline have been used overtime and both have been used as the choice antibiotic for dying off, are relatively inexpensive and available from diverse sources where they are sold with a without prescription in Nigeria. This could be some of the reasons for the 100% resistance by the MRSA isolates against these antibiotics.

The MRSA isolates were highly susceptible against Ciproflaxacin and Gentamycin. Ciproflaxacin, a member of the fluoroquinolones which are newer drugs with mode of action on DNA inhibition are relatively expensive and less available for abuses (26). In addition Gentamycin an aminoglycoside also showed high activity against MRSA which may be as a result of complexity of the aminoglycoside and the route of administration (26). It was concluded that all the MRSA isolates were multi-drug resistant in the current study, based on the assertion by Neyra *et al*., 2014 that 'MDR are bacterial isolates that are resistant to three or more classes of antimicrobial agents'.

V. CONCLUSION

It is concluded that MRSA are present in the nostrils of apparently healthy cattle in fattening lots in Maiduguri, Borno state. The prevalence of MRSA in the study is 21.3%. Ciprofloxacin is most effective antibiotic against MRSA followed by Gentamycin. Therefore, this study recommends the use of ciprofloxacin as an alternative therapy for MRSA infections in the study area.

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