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Vol. 8(27), pp. 2643-2649, 2 July, 2014 DOI: 10.5897/AJMR2014.6855 Article Number: 4997B8545868 ISSN 1996-0808 Copyright © 2014 Author(s) retain the copyright of this article http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization rate among ruminant animals slaughtered for human consumption and contact persons in Maiduguri, Nigeria

Mai-siyama I. B.¹, Okon K. O.^{2*}, Adamu N. B.¹, Askira U. M.³, Isyaka T. M.⁴, Adamu S. G.¹ and Mohammed A.¹

¹Department of Veterinary Public and Preventive Medicine, University of Maiduguri, Borno State, Nigeria.
²Department of Medical Microbiology, Federal Medical Centre, Makurdi, Benue State, Nigeria.
³Department of Medical Laboratory Sciences, College of Medical Sciences, University of Maiduguri, Borno State,

Nigeria.

⁴Department of Medical Microbiology, College of Medical Sciences, University of Maiduguri, Borno State, Nigeria.

Received 25 April, 2014; Accepted 13 June, 2014

This study determined the methicillin-resistant Staphylococcus aureus (MRSA) colonization rate among ruminant animals slaughtered for human consumption and contact persons. Nasal and milk product samples were collected from the main abbatoir in Maiduguri and analyzed using standard bacteriological procedures. A total of 510 samples were analyzed, 87 (17.1%) S. aureus isolates were identified, 33 (34.6%) MRSA and 54 (65.9%) methicillin-sensitive Staphylococcus aureus (MSSA) isolates were detected. 19 (21.8%) MRSA and 17 (19.5) MSSA strains were recovered from cattle, 10 (12.5%) MRSA isolates were recovered from the Red Bororo cattle breed and 12 (17.1) MSSA from carmelius dromedarius. In overall antimicrobial susceptibility pattern, MRSA isolates exhibited multidrug resistance pattern, moderate susceptibility to ciprofloxacin (42.2%), tobramycin (36.4%), amikacin (36.4%), streptomycin (42.2%), while majority of MSSA isolates demonstrated high sensitivity pattern (>70%). Six (6.9%) S. aureus isolates (2 MRSA from cattle and 4 MSSA from sheep) exhibited inducible phenotype. In conclusion, the study findings reveal a relatively high MRSA colonization rate and unique resistance pattern, particularly to topical antimicrobial agents (fusidic acid, mupirocin) that are not routinely used in veterinary medical practice in the study area. The study findings provides a baseline epidemiological information for better understanding of MRSA infections in human and veterinary medicine including foods of animal origin.

Key words: Methicillin-resistant Staphylococcus aureus (MRSA), colonization rate, ruminant animals, contact persons, abattoir.

INTRODUCTION

Staphylococcus aureus is one of the bacterial pathogens that colonize the anterior nares of human and different

animals, including farm animals (Wertheim et al., 2004; Weese and Duijkeren, 2009). The *S. aureus* pathogenicity

is attributable to the expression of wide range of extracellular toxins and virulence factors responsible for superficial and systemic infections (Jarraud et al., 2002; Francis et al., 2005). Since the first report of methicillin resistant S. aureus (MRSA) strain in 1961, the pathogen had attracted public health attention worldwide because it was identified as the major causative agent of hospital associated infections responsible for the significant proportion of hospital admission. Subsequently, MRSA strains was detected in the community setting termed as CA-MRSA, with distinctive predisposing risk factors and molecular characteristics (Francis et al., 2005). The epidemiological trend of MRSA continued to evolve in its phenotypic/molecular characteristics, predisposing risk factors and associated clinical conditions presentations/complications.

In the last decade, the emergence of MRSA among livestock, particularly pigs and other ruminant animals had added different epidemiological dimension to the understanding of the infection. These livestock are seen as a reservoir, capable of transmitting the pathogens to human or vice versa in the community (Vanderhaeghen et al., 2010; Graveland et al., 2011). However, the transmission of LA-MRSA will depend on the level of contact between human and animals, while the introduction into food chain will be through colonized animals (Kock et al., 2009; Lozano et al., 2011).

In Nigeria, available data on the LA-MRSA colonization rate, predisposing risk factors and transmission between humans and animals are scare. Therefore, epidemiological information on LA-MRSA pathogens is imperative, as it will provide a baseline information needed for better understanding of the possible transmission means and its overall public health implications in the community.

Maiduguri is the administrative capital of Borno state located on latitude 9°.45' and 11°.50' North and longitude 10°.05' and 13°. 05' North. It lies within the semi-arid zone, boarded by 3 republics of Niger, Chad and Cameroon. Livestock rearing, particularly ruminant animals are done at the larger scale for economic purposes, while smaller scale involves domestication of animals within the compound which allows close proximity with human population. The state remains the major source of ruminant animals, transported to other parts of Nigeria in Nigeria. In the northeastern Nigeria, few epidemiological data on MRSA in human infections has been published (Okon et al., 2013), but there are no similar data on veterinary infections. Considering the geographical location, the intra-and inter-human activities and rearing and movement of large number of ruminant animals within and outside the state, all these activities constitute major predisposing risk factors for emergence

of resistant pathogens such as LA-MRSA isolates. The public health concern of LA-MRSA continued to heighten worldwide because of possible transmission to human from animals or vice versa and introduction into food chain. Based on this epidemiological information, we decided to assess the MRSA colonization rate among ruminant animals slaughtered at the major abattoir in Maiduguri, and the contact persons.

MATERIALS AND METHODS

The study samples were collected at the Maiduguri Metropolitan Council major abattoir, Maiduguri between January and June 2012. The University of Maiduguri, Faculty of Veterinary Medicine Institutional Review Board and Borno State Veterinary Department attached with abattoir approved the study and the sample collection. Demographic information of the animals were obtained from animal owners and the information entered to the study questionnaire. Demographic information, the type and breed of the animal, antibiotic/local remedy use and grazing pattern are presented in Table 1. Local remedy was defined as concoction prepared with herbs and local materials used by animals owner for treatment of animals infections. The author (IBM) was trained at the University of Maiduguri Veterinary Teaching Hospital in nasal and milk samples collection from ruminant animals and at University of Maiduguri Teaching Hospital for nasal sampling of contact persons. Contact person was defined as persons with close contact with ruminant animals, in this study abbaitor workers are classified as contact persons. A total of 510 samples were collected, the breakdown is as follows: 102 camels, 145 sheep, 113 cattle, 113 goat, 23 milk and 14 contact persons.

Sampling and bacteriological identification procedures

Sterile cotton-tipped swabs were inserted into the inner nasal septum of anterior nare of ruminant animals/contact persons, rubbed several times, removed, capped and labeled appropriately. The milk products were collected by cleaning the udder teat with 70% alcohol, the milk was expressed gently into the study labeled sterile universal bottle, and immediately transported to the laboratory for analysis. The swabs/milk products were inoculated onto blood (BA) and mannitol salt agar (MSA) plates, incubated at 37°C for 24 h. Suspected *S. aureus* colonies with heamolysis on blood agar plates and yellowish appearance on mannitol salt agar plates were further analyzed using standard procedures: colonial morphology, Gram reaction, catalase, tube coagulase and DNase test (Cheesborough, 2006).

Antibiotics susceptibility test

Antimicrobial susceptibility test was determined by disc diffusion method using Mueller-Hinton agar plates accordingly to CSLI (2006) guidelines. The following antibiotic discs (manufactured by Oxoid, UK) were tested, penicillin (PEN), ciprofloxacin (CIP), tobramycin (TOB), kanamycin (KAN), amikacin (AMK), streptomycin (S), tetracycline (TET), trimethoprim (TRIM), erythromycin (ERY), clindamycin (CLD), rifampicin (RF), fusidic acid (FA), mupirocin (MUP) (5 mg, 200 mg). The zone of inhibition around the discs were

*Corresponding author. E-mail: okonkenneth@gmail.com.

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Sample	Number analyzed (%)	Number positive for S. aureus (%) p-value
Milk	23 (4.5)	7 (8.0)
Cattle	113 (22.2)	36 (41.4)
Camel	102 (20.0)	18 (20.7) 0.001
Goat	113 (22.2)	7 (8.0)
Sheep	145 (28.4)	9 (10.3)
Contact persons	14 (2.7)	10 (11.5)
Exposure to antibiotic	111 (21.8)	
Local remedy	385 (75.5)	0.01
Extensive grazing	392 (76.2)	
Intensive grazing	104 (20.4)	
MRSA	33 (37.9)	0.01
MSSA	54 (62.1)	

Table 1. Demographic variables and bacteriological data of sample analyzed.

Table 2. The Frequency of occurrence of S. aureus (MRSA, MSSA) strains within the sampled analyzed.

S. aureus	Milk	Cattle	Camel	Goat	Sheep	Contact persons	Total
MRSA [*]	2 (2.3)	19 (21.8)	2 (2.3)	3 (3.4)	4 (4.6)	3 (3.4)	33 (37.9)
MSSA [*]	5 (5.7)	17 (19.5)	16 (18.4)	4 (4.6)	5 (5.7)	7 (8.0)	54 (62.1)
Total	7 (8.0)	36 (41.4)	18 (20.7)	7 (8.0)	9 (10.3)	10 (11.5)	87 (100)

*Statistical significant difference was observed among the isolates and samples.

measured and interpreted as sensitive, intermediate and resistant according to CSLI breakpoint. Methicillin resistance expression was detected by disc diffusion method using oxacillin and cefoxitin discs.

The D-test for demonstration of inducible phenotype was carried out as previously described by Fiebelkorn et al. (2003), in which the erythromycin and clindamycin discs were placed at 12-14mm apart and inducible phenotype (iMLSB) was indicated by flattening of the clindamycin zone adjacent to the erythromycin discs. *S. aureus* ATCC 26923 was used as a standard control strain.

Data analysis

Data was analyzed by using SPSS version 16.0, the values were expressed as frequency of occurrence and percentages. Comparison of the demographic variables was determined by Chi-square test, p values <0.05 was considered statistically significant.

RESULTS

A total of 510 samples were analyzed, 87 (17.1%) *S. aureus* isolates were identified: 33 (37.9%) MRSA and 54 (62.1%) methicillin-sensitive *S. aureus* (MSSA) isolates. Demographic variables of the *S. aureus* isolates are presented in Table 1, 41.4% (n=36) *S. aureus* isolates were recovered from cattle, 20.7% (n=18) from camel and 11.5% (10) from contact persons (p<0.001). 111 (21.8%) ruminant animals were exposed to antibiotics as compared to 385 (75.5%) with local remedy (p<0.01). 392

(76.2%) practiced extensive grazing as compared to 104 (20.4%) intensive grazing (p<0.01).

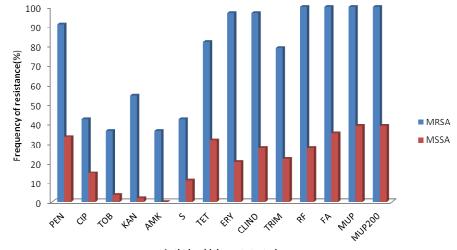
Table 2 present the distribution of *S. aureus* (MRSA, MSSA) isolates according to samples analyzed, 19 (21.8%) MRSA isolates were recovered from cattle, 4 (4.6%) sheep, 3 (3.4%) goat, 2 (2.3%) milk and 3 (3.4%) contact persons, while the frequency of occurrence of MSSA isolates were as follows, 17 (19.5%), 5 (5.7%), 4 (4.6), 5 (5.7%) and 7 (8.0%) (p<0.001). High *S. aureus* colonization rate was recorded among two ruminant animals (Table 3), 10 (14.3%) MRSA isolates were recovered from Red bororo cattle breed as compared to 12 (17.1%) MSSA strains from Carmelius dromedarius (Table 3) (p<0.01).

The antimicrobial susceptibility pattern of *S. aureus* strain tested is Depicted in Figure 1, MRSA strains demonstrated high resistance rates to penicillin (90.9%), fusidic acid (100%), mupirocin (5 mg and 200 mg) (100%), tetracycline (81.8%), clindamycin (97.0%), erythromycin (90.9%), trimethoprim (78.8%), rifamipicin (100%), moderate resistance rate with ciprofloxacin (42.4%), tobramycin (36.4%), amikacin (36.4%) and streptomycin (42.2%), respectively. While majority of the MSSA isolates (>60%) demonstrated susceptibility to all agents tested.

Antimicrobial resistance pattern of *S. aureus* strains according to source of the samples analyzed (Figure 2), showed high susceptibility rate with *S. aureus* strains

Ruminant animal	MRSA (%)	MSSA (%)	Total
SokotoGudali	-	2(2.9)	2(2.9)
Red Bororo	10(14.3)	5(7.1)	15(21.4)
White Fulani	7(10.0)	7(10.0)	14(20.0)
Adamawa White	3(4.3)	3(4.3)	6(8.6)
Muturu	1(1.4)	2(2.9)	3(4.2)
Carmeliusdrumedarium	2(2.9)	12(17.1)	18(25.7)
Yankasa	2(2.9)	1(1.4)	3(4.3)
Ouda	2(2.9)	4(5.7)	6(8.6)
Balami	-	-	-
Sahel	2(2.9)	4	6(8.6)
Sokoto Red	1(1.4)	0	1(1.4)
Total	30(42.9)	40(57.1)	70(100)

 Table 3. The S. aureus strains (MRSA and MSSA) colonization rate of ruminant animals breed sampled.



Antimicrobial agents tested

Figure 1. Antimicrobial resistance pattern of S. aureus (MRSA and MSSA) strains (%).

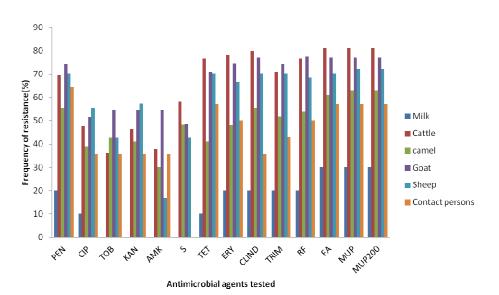


Figure 2. Antimicrobial resistance percentage of S. aureus from samples analyzed.

from the milk products, moderate resistance to ciprofloxacin, tobramycin, kanamycin, amikacin, trimethoprim and clindamycin for strains from contact person, camel, cattle sheep. High resistance to penicillin, tetracycline, erythromycin, clindamycin, trimethoprim, fusidic acid, rifampicin, and mupirocin was observed with *S. aureus* strains from cattle, goat and sheep. Six *S. aureus* strains (2 MRSA from cattle, and 4 MSSA from sheep) demonstrated inducible phenotype.

DISCUSSION

The epidemiology of MRSA infections continued to evolve, with different characteristic patterns and associated clinical complications are reported. Adequate knowledge on the predisposing risk factors and infection control approach within the hospital and community setting is of utmost importance. The emergence of LA-MRSA has added additional epidemiological dimension to the understanding of MRSA infections. In developing countries, particularly in sub-saharan Africa with paucity of epidemiological data on MRSA infections, future data are needed from both human and animal population. To the best of our knowledge, this is first report of MRSA colonization rate among ruminant animals slaughtered for human consumption and contact persons from Maiduguri, Nigeria. Main findings of our study are, (i) MRSA colonization rate among the ruminant animals and contact person, (ii) antimicrobial susceptibility pattern of S. aureus isolates and (iii) the demographic variables associated with MRSA colonization. Therefore, the findings have shed light on LA-MRSA colonization in the study area and its public health and food safety (Lee, 2003; Zschock et al., 2005).

In this study, the S. aureus colonization rate was 87 (17.1%), 33 (37.9%) MRSA isolates and 54 (62.1%) MSSA isolates were detected. The low MRSA colonization rate as compared to MSSA pattern is similar to the pattern reported in other studies (Alzohairy, 2011; Gharsa et al., 2012). MRSA colonization rate differs with the animals sampled and geographica location, in study conducted in Saudi Arabia high MRSA colonization rate was recorded among camels (35.5%) and cattle, 19 (21.8%) as compared to 21.8% in cattle and 4.6% in sheep recorded in our study. While varied rate had been reported in other studies, 44.8% in France and 29% in Tunisia (Vautor et al., 2005; Gharsa et al, 2012). The low MRSA colonization rate of 4.6% is similar to the level reported in Poland (Stastkova et al., 2009). Apart from the fact that high MRSA and MSSA colonization rate was recorded among the cattle in this study, it is also of public health concern, because the cattle constitute the highest number of ruminant animals reared within the community in the study area and the major source of animal proteins. Reason for public health concern are, (i) possible transmission and dissemination of the MRSA isolates

could occurs through the level of contact that include close proximity through rearing and domestication, (ii) nasal dropping during movement within the community and (iii) contamination of meat and milk products by colonized handlers. Studies have reported that MRSA colonization of cattle posed a potential risk of up to 60% transmission to the contact persons (Lee, 2003; Juhasz-Kaszanyitzky et al., 2007).

In this study, the MRSA colonization rate among contact persons was 3.4%, this level is lower when compared with the level reported in other studies that assessed the level of contact as a predisposing risk factor for colonization. In these studies, the overall MRSA colonization rate was 6.5% level reported among veterinary personnel, 16% among veterinarian handling large animals and 4.4% among those handling small animals (O'Mahony et al., 2005; Simoons-Smit et al., 2000; Beth et al., 2006; Hanselman et al., 2006). Nevertheless, the level of MRSA colonization rate among contact persons varied with geographic location, type of animals and culture methods employed in the studies (Vanderhaeghen et al., 2010; Graveland et al., 2011).

The MRSA contamination of milk and dairy products are known to be through infection like masititis or the hands of the farmers. The level of milk and dairy products contamination with MRSA isolates varies with geographical location, as low contamination level is reported in European countries, the USA and Canada in contrast to high level reported in Asia and Africa (Pexara et al., 2013). In our study, the MRSA colonization rate recovered from milk product sampled was 2.3%, this level is lower as compared to the level reported in other similar studies, 17.9% in Iran (Alian et al., 2012), 60% in Ethopia (Daka et al., 2012), 36% in Jos, Nigeria (Suleiman et al., 2012) and 6% in South Africa (Ateba et al., 2010). Variation in the colonization rate might be due to the animal production systems, presence of multiple animal species within the same area that could facilitate transmission and dissemination of the pathogens and the animals handling processes particularly during the milking (Vanderhaeghen et al., 2010; Graveland et al., 2011).

We observed that the *S. aureus* isolates showed 3 distinctive patterns: low, moderate and high resistance pattern, with the MRSA exhibiting multidrug resistance pattern. The interesting finding of this study is, the MRSA isolates showed high resistance pattern to topical antibacterial agents (fusidic acid, mupirocin), that are clinically used for MRSA decolonization/decontamination in human and veterinary medicine. These topical antibacterial agents are not routinely used in veterinary practices in the study area. Therefore, the reason for such resistance pattern by the *S. aureus* isolates remain unclear. Future research studies are needed to provide insight, through molecular characterization of resistance genes. Although, some studies have reported fusidic acid and mupirocin-resistant *S. aureus* strains in human and

animals population, which varied with different geographical locations (Udo et al., 2001; Chen et al., 2010).

In Nigeria, the frontline antimicrobial agents routinely used in human and veterinary medicine are tetracycline, erythromycin, penicillin and some quinolones. In this study, of the antimicrobial agents tested, both MRSA and MSSA isolates demonstrated high degree of sensitivity to some aminoglycosides like tobramycin, amikacin and streptomycin. This pattern revealed that these agents are not only still efficacious for staphylococcal infections treatment and management, but also as alternate option for treatment of livestock infection due to multidrug resistant S. aureus strains in veterinary settings. In addition, 6 (6.9%) S. aureus strains (2 MRSA from cattle and 4 MSSA from sheep) demonstrated inducible phenotype, the pattern is consistent with other studies which predominate in MSSA AS compared to MRSA (Alzohairy, 2012: Schreckenberger et al., 2004: Levin et al., 2005). As obtainable worldwide, macrolides are frontline antibiotics widely used for the treatment of human and animal infections. Extensive usage of these antibiotics results in selection of resistant bacteria and genetic determinants of resistance can be transmitted from animals to humans via foodstuffs (Perreten et al., 1998; Schlegelova et al., 2004). The D-test is used to demonstrate the constitutive and inducible phenotype, and determination of possible chemotherapeutic failure (Levin et al., 2005).

surveillance studies Although, are encouraged worldwide to provide epidemiological data on LA-MRSA, but there are limitation, particularly in comparison with epidemiological data. These limitations include lack of standardization in the methodology employed, in addition, Staphylococcus spp., like Staphylococcus intermedius, Staphylococcus schlieferi, Staphylococcus hvicus. Staphylococcus delphini and Staphylococcus pseudointermedius, produced positive tube coagulase result that may be detected as MRSA, particularly in lowresource laboratory in which analysis are based on phenotypic characterization (Morgan, 2008).

Conclusion

Based on the findings of this study, we can state that MRSA colonization rate in ruminant animals and contact persons might be assumed to be relatively high for geographical location without no pre-existing epidemiological data for comparison and the resistance pattern is of public health concern. The reason being that these resistant strains and genes can be transmitted and disseminated between human and animals, and subsequently into the food chain. The scenario could worsen in this area if appropriate attention is not paid.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

We acknowledge the assistance of Dr. E. E. Udo of University of Kuwait, who graciously provided us with the antibiotics, and Messer Mustapha and Saleh of Department of Medical Microbiology, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria for their assistance in the preparation of the media and reagents.

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