International Journal of Public Health and Epidemiology ISSN: 2326-7291 Vol. 3 (8), pp. 054-058, August, 2014. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Occurrence of vancomycin-resistant *staphylococcus aureus* (VRSA) in fresh and fermented milk in Nigeria: A preliminary report

Gali Abaka Umaru[1*], Junaidu Kabir[2], Veronica J. Umoh[3], Mohammed Bello[2], and Jacob K. P. Kwaga[2]

[1*]Department of Animal Health, College of Agriculture, P.M.B.1025, Jalingo-660001, Taraba State, Nigeria.
[2]Faculty of Veterinary Medicine, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.
[3]Faculty of Science, Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Accepted 7, April 2014

A study was conducted to determine the occurrence of vancomycin resistant *S. aureus* (VRSA) in milk in Nigeria. Fourty seven *S. aureus* were isolated from the 372 milk samples examined, out of which 20 (5.4%) were vancomycin-resistant. Fifteen (4%) of the VRSA were isolated from raw milk, 4 (1.1%) from bulk milk and 1 (0.3%) from pasteurized milk. No significant difference (P > 0.05) between the occurrence among the study variables was observed, . No VRSA occurred in the yogurt and 'kindirmo' samples examined. The decrease in the occurrence of the pathogen from raw milk to pasteurized milk and its absence in yogurt and 'kidirmo' suggest that pasteurization and fermentation may have eliminated most of the organisms. The resistance profiles of the VRSA isolates to other antibiotics showed high resistance to penicillin (100%), tetracycline (85%), amoxicillin (65%), methicillin (40%) and oxacillin (40%), but low to amikacin (5%) and sulphamethoxazole/trimethoprim (10%). None of the isolates were resistant to chloramphenicol and ciprofloxacin. The multiple antibiotic resistance index result revealed that 80% of the VRSA were resistant to 3 or more antibiotics. The presence of VRSA coupled with the multple resistance patterns of these isolates are of great concern and also of public health significance. Therefore more studies on the epidemiology of this pathogen in foods are required.

Key words: Vancomycin-resistant *Staphylococcus aureus* (VRSA), Fresh Milk, Fermented Milk, Multiple Antibioic Resistance Index (MARI), Preliminary report, Nigeria.

INTRODUCTION

In an attempt to isolate methicillin-resistant *S. aureus* (MRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria, significant percentage of the isolated *S. aureus* were discovered to be vancomycin resistant following antibiotic resistance testing. This is of great concern since vancomycin is the drug of choice after the failure of methicillin, coupled with the fact that vancomycin resistance is rarely reported even in clinical cases. This report, therefore serves as preliminary information on the occurrence of vancomycin resistant *Staphylococcus aureus* (VRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria. *Staphylococcus aureus* (*S. aureus*) is one of the bacteria that have developed resistance to most classes of antibiotics including methicillin **(**Weigel et al., 2007**)**. Vancomycin then became a drug of choice for the treatment of infections with *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA), until in 1996 when the first Vancomycin-resistant *Staphylococcus aureus* (VRSA) was isolated from a Japanese patient **(**Hiramatsu, 2001**)**. Subsequently, it was isolated in USA, France, Korea, South Africa, and Brazil **(**Hiramatsu, 2001; Weigel et al., 2007**).** Vancomycin-resistant *Staphylococcus aureus* (VRSA) is therefore, a strain of *Staphylococcus aureus* that is resistant to the antibiotic glycopeptide, vancomycin **(**Chang et al., 2001**).** The resistance is acquired by mutation and thickening of cell

*Corresponding author.E-mail: drghaliumaru@yahoo.com.

wall due to accumulation of excess amounts of peptidoglycan.

In Nigeria, resistant pathogens including VRSA have been detected in both hospital and non-hospital sources **(**Olayinka et al., 2006; Onanuga et al., 2006**)**, but however literature is scarce on the prevalence of both MRSA and VRSA in foods (Umaru et al., 2011). The current information regarding the prevalence of MRSA in fresh and fermented milk in Zaria and Kaduna, Nigeria is the one reported by Umaru et al. (2013) in which *mec* A positive MRSA were detected with a prevalence of 4.8% (Umaru et al., 2013). This report therefore, provides preliminary information on the occurrence of VRSA in milk in Nigeria.

MATERIAL AND METHODS

Study Areas and Samples Collection

The study was conducted in Kaduna and Zaria, Kaduna State, Nigeria and covered villages and herds of cattle supplying two peri-urban dairy groups (MILCOPAL in Kaduna and NAPRI in Zaria). Three hundred and seventy two (372) milk samples were collected during the study period. This comprised 300 raw milk (collected directly from individual cows during milking), 18 raw pooled milk (collected after all the milk from individual cows were pooled in one container), 17 pasteurized milk and yogurt (collected from the two yogurt processing plants) and 20 'kindirmo' (locally fermented milk) collected from the local Fulani processors. All the samples collected were placed on ice and transported to the laboratory were bacteriological examinations were conducted.

Isolation of Staphylococci from Milk Samples

All the samples were cultured on Baird Parker Medium (Oxoid, Basingstoke, England), which was prepared according to the manufacturer's instruction, and incubated aerobically at 37^{o} C for 24 hours (Tamagnini et al., 2006).

Pure colonies of each isolate, which were typically grey-black with narrow white margin surrounded by zone of clearing were obtained by streak plating and inoculated onto nutrient agar slants and stored prior to identification (O' Brieen et al., 2009).

Identification of the *Staphylococcus Aureus*

The identifications were done using colony morphology, gram staining characteristics and biochemical tests namely; catalase, coagulase, DNase and sugar fermentation (Cheesbrough, 2003; Japoni et al., 2004). *S. aureus* ATCC 3359 strain was used as a positive control.

Antimicrobial Resistance of Coagulase Positive *S. Aureus*

Antimicrobial resistance was performed by Kirby- Bauer disk diffusion method as recommended by Clinical Laboratory Standards Institute (CLSI) (Bauer et al., 1996; CLSI, 2006). All isolates were grown in Brain Hearth Infusion broth (Biotech Laboratories, United Kingdom) and incubated at 37^{o} C for 6 hours until the turbidity equivalent to 0.5 McFarland standards was achieved. The isolates were then swabbed onto Mueller Hinton Agar (Amershan, England) before the application of the antimicrobial discs. The isolates were tested against a panel of twelve (Hanselman et al., 2006) antibiotics with the following concentrations: amikacin (30 µg), amoxicillin (30 µg), chloramphenicol (12 µg), ciprofloxacin (5 µg), erythromycin (5 µg), gentamicin (10 µg), methicillin (10 µg), oxacillin (1 µg), penicillin (10 IU), sulphamethoxazole/trimethoprim (5 µg), tetracycline (30 µg) and vancomycin (30 µg) (Oxoid, England). Zones of inhibition (ZI) were measured and recorded after 24 hours of incubation at 35^{o} C, and were interpreted according to the guidelines of the CLSI (CLSI, 2006). The isolates that were resistant to vancomycin (ZI < 15mm) were regarded as vancomycin-resistant *Staphylococcus aureus* (VRSA) while those that were susceptible were termed vancomycin-susceptible. *S. aureus* ATCC 3359 strain was used as a positive control.

Multiple Antibiotic Resistance Index (Mari)

The multiple resistance indexes were determined for each VRSA isolates by dividing the number of antibiotics to which the isolate is resistant to by the total number of antibiotics tested (Olayinka et al., 2005).

Data Analysis

Data obtained from the study were analyzed statistically using Statistical Package for Social Sciences (SPSS) Version 13 software. Frequencies were obtained and percentages for study variables were calculated. Chi-square and Fisher's exact tests at 5% level of confidence were used to perform categorical comparisons and determination of significance. A P value < 0.05 was considered significant for all comparisons.

RESULTS

The results obtained from the study showed that 47 of the 372 milk samples examined were positive for coagulase positive *S. aureus* while 20 were vancomycin resistant. This gave a prevalence of 5.4% of VRSA (Table 1). The prevalence was significantly higher (P < 0.05) in Kaduna

Table 1. Prevalence of staphylococci and vancomycin-resistant *Staphylococcus aureus* (VRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria.

Location	No. of samples	No.(%) Coagulase negative staphylococci	No.(%) Coagulase Positive staphylococci	No. (%) VRSA
Kaduna	196	49(25.0)	27(13.8)	7(3.6)
Zaria	176	31(17.6)	20(11.4)	13(7.4)
Total	**372**	**80(21.5)**	**47(12.6)**	**20(5.4)**

Table 2. Prevalence of staphylococci and vancomycin-resistant *Staphylococcus aureus* (VRSA) according to the type of sample examined in Zaria and Kaduna, Nigeria.

Type of sample	No. of sample tested	No.(%) Coagulase negative staphylococci	No.(%) Coagulase Positive staphylococci	No. (%) VRSA
Raw milk	300	75(20.2)	36(9.7)	15(4.0)
Bulk milk	18	3(0.8)	9(2.4)	4(1.1)
Pasteurized Milk	17	1(0.3)	1(0.3)	1(0.3)
Yogurt	17	0(0.0)	0(0.0)	0(0.0)
Kindirmo*	20	0(0.0)	1(0.3)	0(0.0)
Total	**372**	**80(21.5)**	**47(12.6)**	**20(5.4)**

* Local processors

(7.4%) than in Zaria (3.6%). The distribution of the VRSA among the different types of samples examined is shown in Table 2. Fifteen (4 %) of the VRSA were isolated from raw milk, 4 (1.1 %) from bulk milk and only 1 (0.3 %) from pasteurized milk. No significant difference ($P > 0.05$) exists between the occurrences among the study variables. No VRSA was isolated from yogurt and 'kindirmo' samples examined (Table 2).

Antibiotic Resistance Profile of The Vrsa Isolates

The resistance patterns of the 20 VRSA isolates are, Amikacin (5%), amoxicillin (65%), erythromycin (50%), gentamicin (8%), methicillin (40%), oxacillin (40%), penicillin (100%), sulphamethoxazole/trimethoprim (10%) and tetracycline (85%). None of the VRSA isolate was resistant to both chloramphenicol and ciprofloxacin (Table3).

Table 3. Antimicrobial resistance profile of *S. aureus* and VRSA isolated from fresh and fermented milk in Zaria and Kaduna, Nigeria.

Antibiotic	Coagulase positive *S.aureus* n= 47	VRSA n= 20
Amikacin	1 (2.1)	1 (5)
Amoxicillin	21 (44.7)	13 (65)
Chloramphenicol	2 (4.3)	0 (0)
Ciprofloxacin	0 (0)	0 (0)
Erythromycin	15 (31.9)	10 (50)
Gentamicin	12 (25.5)	8 (40)
Methicillin	18 (38.3)	8 (40)
Oxacillin	22 (46.8)	8 (40)
Penicillin	47 (100)	20 (100)
Sulphamethoxazole/trimethoprim	3 (6.4)	2 (10)
Tetracycline	15 (31.9)	17 (85)
Vancomycin	20 (42.6)	

N. B. Numbers in parenthesis indicate percentages.

Table 4. Multiple resistance indexes of vancomycin-resistant *Staphylococcus aureus* (VRSA) isolated from Milk samples in Kaduna and Zaria, Nigeria.

MAR Index	No. of isolates	% of isolates
0.1	1	5
0.2	2	10
0.3	5	25
0.4	1	5
0.5	11	55
Total	**20**	**100**

Determination of the multiple antibiotic resistance index (MARI) of the isolates shows that 80% were resistant to 3 or more antibiotics with 55% of the isolates having the highest MARI of 0.5 (Table 4).

DISCUSSION

Methicillin resistant *Staphylococcus aureus* (MRSA) is common in humans in both hospital and non hospital sources across the world (Japoni et al., 2004; Klevens et al., 2007). Later, MRSA was detected in companion animals (Cuny et al., 2010; Huber et al., 2010) and most recently in foods (Tüirkyilmaz et al., 2010; Bhargava et al., 2011; Umaru et al., 2013). However, a study on the prevalence of MRSA in foods in Nigeria is scarce and hence a study was carried out to determine the occurrence and prevalence in fresh and fermented milk in Zaria and Kaduna, Nigeria. Following antibiotic susceptibility testing, significant percentage of the *S. aureus* and MRSA were found to be resistant to vancomycin. This is surprising and of concern since vancomycin is rarely used in the treatment of animals in these areas, coupled with the fact that vancomycin resistance is rarely reported even in clinical cases. Infections with and prevalence of vancomycin-resistant *Staphylococcus aureus* (VRSA) have been documented among ill and healthy humans across the world including Nigeria **(**Hiramatsu , 2001; CDC, 2004; Olayinka et al., 2005; Onanuga et al., 2006; Weigel et al., 2007; Chang et al., 2009). Information is lacking on the occurrence of VRSA in foods, which may serve as potential sources of infections to humans.

Although, polymerase chain reaction (PCR) was not conducted to detect *van* A gene, which is regarded as the gold standard for vancomycin resistance, susceptibility testing to 30 µg vancomycin discs (Oxoid, England) was used as recommended by Clinical Laboratory Standards Institute (CLSI, 2006).

In this study, an overall prevalence of 5.4% was recorded. Presently, there is no data on the prevalence of VRSA in foods in the study areas or in other locations for comparison with this study. The only available data on VRSA in the study area are the ones reported by Olyinka et al. (2005) and Onanuga et al. (2005) with the prevalence rates of 57.7% and 89.2% at University Teaching Hospital, Zaria and among healthy women in Zaria respectively. The samples used for their studies were from humans, and which preclude any meaningful comparison with the present study. The occurrence of the VRSA among the different types of samples examined showed that 15 (4.0 %) were isolated from raw milk, 4 (1.1 %) from bulk milk and only 1 (0.3 %) from pasteurized milk. No VRSA was isolated from yogurt and 'kindirmo' samples examined (Table 2). The low occurrence of the VRSA in the pasteurized milk and the total absence in yogurt and 'kidirmo' samples suggest that the efficient pasteurization and fermentation procedures may have eliminated most of the pathogens. These processes may therefore, be the recommended measures for the elimination of VRSA in milk and also controlling its spread and transmission to humans. The occurrence of VRSA in these study areas where vancomycin is not being used in animals may suggest either secondary contamination by handlers or processors or direct transmission from humans to the animal. Studies are required to prove this. Therefore exposure to vancomycin may not necessarily be a factor in the acquisition of resistance to vancomycin.

The resistance profiles of the VRSA isolates to other antibiotics showed high frequency of resistance to penicillin (100%), tetracycline (85%), amoxicillin (65%), methicillin (40%) and oxacillin (40%), but low to amikacin (5%) and sulphamethoxazole/trimethoprim (10%). All the VRSA isolates were susceptible to chloramphenicol and ciprofloxacin.

This connotes that amikacin, sulphamethoxazole/trimethoprim, chloramphenicol and ciprofloxacin could be reliably used in the treatment of VRSA infections due to these strains in these areas. Multiple resistance index results showed that 80% of the isolates were resistant to 3 or more antibiotics, a phenomenon known as multiple drug-resistance patterns and indicating how dangerous these isolates could be. As with many studies involving human isolates, MRSA were reported to be highly or totally resistant to vancomycin (Chang, 2003), while in some cases reduced susceptibility was observed (Bukhar, 2004; Mehmood and Usman, 2007; Kaleem et al., 2012).

CONCLUSION AND RECOMMENDATIONS

Methicillin-resistant *Staphylococcus aureus* (MRSA) was initially regarded as a hospital pathogen (Hanselman et al., 2006), but was later discovered outside the hospital environment (Klevens et al., 2007; Cuny et al., 2010), later in animals (Huber et al., 2009; Van Duijkeren et al., 2011; Bhargava et al., 2011), and most recently in foods (Tüirkilmaz et al., 2009; Bhargava et al., 2011; Umau et al., 2013).

The occurrence of VRSA in milk may suggest its presence in other foods and food animals. More studies are recommended to prove this or otherwise. Although VRSA is not common in Nigeria, its isolation in milk and the unique multi-drug resistance patterns displayed, calls for more studies and surveillance to forestall spread to humans.

ACKNOWLEDGEMENTS

The authors acknowledge with great thanks the technical assistance rendered by Mallam Yahuza Suleiman Maitala of the Bacterial Zoonoses Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, and the management and staff of MILCOPAL dairy plant in Kaduna and National Animal Production Research Institute (NAPRI), Zaria for their assistance during sample collections.

REFERENCES

Baird-Parker AC (1962). An improved diagnostic and selective medium for isolating coagulase positive staphylococci. J. Appl. Bacteriol. **25:** 12-10.

Bauer AW, Kirby WM, Sheris JKT (1996). Antimicrobial susceptibility testing by a standard single disc method. Amer. J. Clin. Path. **45**: 493-496.

Bhargava K, Wang X, Donabedian S, Zervos M, da Rocja L, Zhang Y (2011). Methicillin-resistant *Staphylococcus aureus* in retail meat, Detroit, Nichigan, USA. Emerg. Infec. Dis. **17**(6): 1135-1137.

Bukhari MH (2000). A laboratory study of susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA). Pakistan J. Med. Sci. **20**: 229-233.

Centers for Disease Control and Prevention (2004). Brief report: Vancomycin-resistant *Staphylococcus aureus*-New York. Morb. Mort. Week. Rep. **53**: 322-333.

Chang S, Sievert DM, Hageman JC, Boulton ML, Tenover FC, Downes FP, Shah S, Rudrik JT, Pupp GR, Brown WJ, Cardo D, Fridkin SK. (2003). Infectious with vancomycin-resistant *Staphylococcus aureus* containing the *Van* A resistance gene. New Eng. J. Med. **348**: 1342-1347.

Cheesbrough M (2002). *District Laboratory Practice in Tropical Countries,* Part 2, Cambridge University Press. pp. 135–162.

Clinical Laboratory Standards Institute (CLSI)., 2006. Performance standards for antimicrobial susceptibility testing. Sixth Informational Supplement. CLSI document M100-S16 [ISBN 1-56238-5887]. Clinical Laboratory Standards Institute, 940 west Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Cuny C, Friedrich A, Kozytska S, Layer F, Nübel U, Ohlsen K, Strommenger B, Walther B, Wieler L, Witte W (2010). Emergence of methicillin-resistant *Staphylococcus aureus* in different animal species. Int'l. J. Med. Microbiol. **300:** 109-117.

Hanselman BA, Kruth SA, Rousseau J, Low DE, Willey BM, Mcgeer A, Weese JS (2006). Methicillin-resistant *Staphylococcus aureus* colonization in veterinary personnel. Emerg. Infec. Dis. **12**: 1933-1938.

Hiramatsu K (2001). Vancomycin-resistant *Staphylococcus aureus*: A new model of antibiotic resistance. Lancet Infec. Dis. **3**: 147-255.

Huber H, Koller S, Giezendanner N, Stephan R, Zweifel C (2010). Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, livestock, and in food animal origin, Switzerland, 2009. Eurosurveill. **15**(16): 19542.

Japoni A, Alborzi A, Rasouli M, Pourabbas B (2004). Modified DNA Extraction for rapid PCR detection of methicillin-resistant staphylococci. Iran. Biomed. J. **8**(3): 161-165.

Kaleem F, Usman J, Sattar A, Amanat SA, Hassan A, Omair M, Khalid A, Riaz M (2012). Current status of vancomycin susceptibility against methicillin-Resistant *Staphylococcus aureus* (MRSA) strains: A study at two tertiary care hospitals of Pakistan. Afr. J. Microbiol. Res. **6** (33): 6243-6246.

Klevens RM, Morrison MA, Nadle J (2007). Invasive of Methicillin–resistant *Staphylococcus aureus* (MRSA) infections in the United States. J. Am. Med. Assoc. **298**: 1763-1771.

Mehmood A, Butt T, Usman M (2007). A stud on MRSA isolates to detect Reduced susceptibility to vancomycin; A preliminary report. Infec. Dis. J. **16**: 102-104.

O'Brien M, Hunt K, McSweeney S, Jordan K (2009). Occurrence of foodborne Pathogens in Irish Farnhouse cheese. Food Microbiol. **26**: 910-914.

Olayinka BO, Olayinka AT, Onaolapo JA, Olurinola PF (2005). Pattern of resistance to vancomycin and other antimicrobial agents in Staphylococcal isolates in a University teaching hospital. Afr. J. Clin. Expt. Microbiol. **6**: 46-52.

Onanuga A, Oyi AR, Onaolapo JO (2006). Prevalence of MRSA with reduced susceptibility to vancomycin among healthy women in two Nigerian metropolitan cities. Afr. J. Clin.Expt. Microbiol. **7** (3): 205-211.

Tamagnini LM, Sousa GB, Gonzalez RD, Bude CE (2006). Microbiological Characteristics of Crottin goat cheeses made in different seasons. Small Rum. Res. **66**: 175-180.

Tüirkyilmaz S, Tekbiyik S, Oryasin E, Bozdogan B (2010). Molecular epidemiology and antimicrobial resistance mechanisms of methicillin resistant *Staphylococcus aureus* isolated from bovine milk. Zoon. Pub. Hlth. **7:** 140-147.

Umaru GA, Kabir J, Adamu NB, Umar YA (2011). A review of emerging methicillin-resistant *Staphylococcus aureus* (MRSA): A growing threat to veterinarians. Nig. Vet. J. **32** (3): 174-186.

Umaru GA, Kabir J, Umoh VJ, Bello M, Kwaga JKP (2013). Methicillin- resistant *Staphylococcus aureus* (MRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria. Intern. J. Drug Res. Tech. **3** (3): 67-75

Umoh VJ (1989). Contamination of fura-da-nono by staphylococci and growth of an enterotoxigenic *Staphylococcus aureus* in fura-a cereal food. Zariya Vet. **4** (2): 53-58.

Van Duijkeren E, ten Horn L, Wagenar JA, de Bruijn M, Laarhoven L, Verstappen K, de Weerd K, Meessen N, Duim B (2011). Suspected Horse-to-Human transmission of MRSA St398. Emerg. Infec. Dis. **17**(16): 1137-1139.

Weigel LM, Donlam RM, Shin DH, Jensen B, Clark NC, McDougal LK, Zhu W, Musser KA, Thompson J, Kohlerschmidt D, Dumas N, Lumberoer RJ, Patel B (2007). High-level of vancomycin- resistant *Staphylococcus aureus* isolates associated with a polymicrobial biofilm. Antimicrob. Agen. Chemother. **5**: 231-238.