Research Article

Nasal Carriage of MRSA among Healthy College Students and Livestock

Kenneth Nnamdi Anueyiagu*, James Jingmang Kopmut, Chrysantus Achi Lagi and Kelly Nkem Okoh

Federal College of Animal Health and Production Technology, NVRI Vom

Abstract | The objective of this study is to determine the rate of nasal carriage of Methicillin Resistant *Staphylococcus aureus* (MRSA) among students in a college of animal health and production and livestock in the College Farm. 160 nasal swab samples taken from 130 students from three schools of the college and 30 livestock comprising sheep, goats, and cows between October 2017 and April 2018. Samples were enriched in peptone water broth, incubated at 37°C for 24 hours, and sub-cultured onto Mannitol Salt Agar and thereafter standard bacterial procedure continued. A total of 83 (51.9%) S *aureus* isolates were identified, 54 (33.8%) MRSA, and 15 (9.4%) Methicillin Susceptible *Staphylococcus aureus* (MSSA). The study showed a statistical association between the prevalence of MRSA in livestock and students. This could lead to cross infection of MRSA from livestock to man and vice versa, hence of public health significance.

Editor | Muhammad Abubakar, National Veterinary Laboratories, Park Road, Islamabad, Pakistan.
Received | October 18, 2019; **Accepted** | February 14, 2020; **Published** | March 17, 2020
***Correspondence** | K.N. Anueyiagu, Federal College of Animal Health and Production Technology, NVRI Vom; **Email:** anueyiagunnamdi@yahoo.com
Citation | Anueyiagu, K.N., J.J. Kopmut, C.A. Lagi and K.N. Okoh. 2020. nasal carriage of MRSA among healthy college students and livestock. *Veterinary Sciences: Research and Reviews*, 6(1): 33-39.
DOI | http://dx.doi.org/10.17582/journal.vsrr/2020/6.1.33.39
Keywords | Nasal carriage, *Staphylococcus aureus*, MRSA, methicillin, livestock, students

Introduction

S. aureus is one of the most significant bacterium associated with the nasal cavity of man and animals causing different pathogenic infection on the skin and soft tissues of both man and animals (Khalid and Ali, 2015).

Duration of treatment depends on the site and severity of the infection. Antibiotic resistance strains of *S. aureus* was first discovered in the year 1880 at Aberdeen, Scotland by surgeon Sir Alexander Ogston in pus from surgical abscesses in a knee joint.

Resistance of *S. aureus* to methicillin, a typical beta-lactam agent, was first reported in 1961. The ability of *S. aureus* to be resistance to methicilin is due to its acquisition of the *mecA* gene, that encodes for a new protein designated PBP2a, belonging to a family of enzymes necessary in building the bacterial cell wall. PBP2a has a very low affinity for beta-lactam antibiotics and confers resistance to methicillin and other beta-lactams (Pantosti, 2007)

Hiffinan in 2015 reported that MRSA strains are the cause of many zoonotic infections in the world today. A population of about 94,360 invasive MRSA infections is diagnosed annually in the US, with 18,650 associated deaths; with approximately 86% of all the invasive MRSA infection are health care associated nosocomial infections.

MRSA infection has been established in literature to affect livestock and are otherwise referred to as Livestock associated-Methicillin Resistant *Staphylococcus aureus* (LA-MRSA). The first

documented account of MRSA in livestock was in a bovine mastitis case in the 1970s. Afterwards, MRSA had been isolated from food animals such as cattle, pigs, horses, and poultry (Aires-de-Sousa, 2017). While Wulf et al. (2006) screened 80 veterinary students and 99 veterinarians for MRSA, Voss et al. (2005) indicated transfer of MRSA from pigs to caretakers.

Using the economic models and findings of Lee et al. (2013), a single community associated with MRSA cost third party payers about $2,277 - $3,200 (which is about N455,400 – N640,000), and society $7,070 - $20,489 (about N1,414,000 –N14,097,800). Depending on the age of the patient, MRSA is peculiar and represent a substantial burden of resistant infections in hospitals (Dilip, 2003).

Direct contact with MRSA positive animals has been reported to be a major risk factor for humans (Grøntvedt et al., 2016) and if not managed properly could lead to outbreaks especially in underdeveloped countries where biosecurity in livestock farms is at the minimal.

The objective of this study was to establish the rate of nasal carriage of MRSA among students in a college of animal health and production, and livestock in the college farm.

Materials and Methods

Study area

This study was designed to cross-sectional survey the nasal carriage of MRSA among students and livestock in an animal health and production college. Approval was obtained from institutional committee on sampling ethics. Samples were collected between October 2017 and April 2018.

Sample collection

A total of about 160 nasal samples were collected from college students and livestock. Ten samples were collected from 13 classes of both National Diploma (ND) and Higher National Diploma (HND) streams from 3 schools (Science and Technology, Animal Health and Production Technology, Agriculture Technology and Environment Science) making a total of 130 samples. While 10 samples each were collected from goats, sheep and cattle respectively. One sterile swab stick, moistened in sterile distilled saline was inserted into the inner nasal septum of anterior nares of students and livestock, rubbed several times according to Hema et al. (2017). Then removed, capped, labeled and transported immediately in Amie's transport media to Microbiology laboratory of Federal College of Animal Health and Production Technology Vom, for analysis.

Bacteriological assay

About 4.5 ml of peptone water was dispensed in 160 empty samples bottles each into which the 160 samples collected were placed for enrichment in peptone water and then incubated for 24 hours at 37 °C. Thereafter, a loopful was inoculated onto Mannitol Salt Agar (MSA) plates and incubated for another 24 hours at 37 °C. Suspected *S. aureus* colonies with yellowish appearance were observed and recorded. Other biochemical tests were carried out according to Cheesebrough (2000).

Susceptibility testing of MRSA isolates

Antibiotic sensitivity test was carried out with organism subculture onto Mueller Hinton Agar, oxacillin test disc placed on it and incubated again for 24 hours at 37 °C.

The diameter of zone of inhibition produced by each of the discs was measured, recorded, and isolates that were ≤ 10 mm were classified as resistant, 11-12 mm as intermediates, and ≥ 13 mm as susceptible based on standard interpretative chart as recommended by the clinical and laboratory start instate (CLSI, 2014).

Data analysis

Chi-Square test of independence, and correlation coefficient were performed to test for the association between the prevalence of MRSA in students and livestock sampled using R commander (Fox, 2019) software.

Results

Among the livestock sampled, sheep had the highest prevalence of 80% of *S. aureus* isolated; among students sampled, 68% those of Animal health and Production Technology had *S. aureus* being the highest group (Table 1). Out of 160 samples collected and analyzed from students and livestock, 83 (51.9%) *S. aureus* were isolated while 80 (44.44%) MRSA were identified.

Table 2 illustrates the association between the prevalence of MRSA in gender of animals and students. All the species had odds ratio of 1 signifying

that the prevalence of MRSA has equal likelihood of occurrence. *S. aureus* isolates from students were more susceptible to antibiotics than isolates of livestock (Table 3). From the susceptibility pattern, the *S. aureus* isolates of students showed more resistance gentamycin than other antibiotics used (Table 4). While Table 5 showed the response of *S. aureus* isolates from sheep to antibiotics according to the susceptibility, intermediate and resistance values of each antibiotics.

Table 1: *Prevalence of S. aureus based on livestock and students.*

Specie	Sample (n)	Positive (n)	Prevalence %
Livestock			
Cattle	10	4	40
Sheep	10	8	80
Goat	10	5	50
Students			
Science and technology	60	27	45.0
Animal health and production technology	50	34	68.0
Agricultural technology and env. science	20	5	25.0
Total	160	83	51.9

Table 2: *Gender prevalence of MRSA isolated from livestock and students.*

Animal	Gender	Sample (n)	Positive (%)	P-value	OR (95% C1)
Cattle	Bull	2	20	1.000	1
	Cows	1	10		1(0.046, 64.797)
Sheep	Ram	2	20	1.000	1
	Ewes	5	50		1(0.088, 6.748)
Goat	Buck	2	20	1.000	1
	Does	3	30		1(0.077, 9.567)
Students	Male	26	20	1.000	1
	Female	39	30		1(0.441, 2.277)

Table 3: *Antibiotics susceptibility of S. aureus from student and ruminant.*

Animal	Sample (n)	Positive (n)	Prevalence %
Cattle	4	0	0
Sheep	8	1	12.5
Goat	5	0	0
Students	66	16	24.24

Discussions

The study shows 50.8% overall prevalence of nasal colonization of *S. aureus* in healthy students in the College of Animal Health and Production Technology with a prevalence of 45%, 68%, and 25% from students of schools of Science and Technology, Animal Health and Production, and Agricultural Technology and Environmental Science respectively. These results showed the students' nasal passage were considerably colonized by this organism. This is consistent with Gulani et al. (2016) with an overall prevalence of 44.3%. The School of Animal Health and Production Technology had the highest level of *S. aureus* prevalence compared to the two other schools. This could be as a result of their regular contact the livestock and environment during farm and clinical practical classes. Zomer et al. (2017) reported a comparatively low prevalence of 0.56% for people who do not have professional contact with livestock which is similar to low prevalence of students of Agricultural Technology and Environmental Science respectively.

Table 4: *Prevalence of antibiotics resistance among students.*

Antibiotics	Susceptible (%)	Intermediate (%)	Resistance (%)
Rifampicin	38 (18.63)	4 (8.16)	8 (7.34)
Gentamycin	16 (7.84)	N/A	34 (31.19)
Erythromycin	21 (10.29)	7 (14.29)	22 (20.18)
Ciprofloxacin	25 (12.25)	15 (30.61)	10 (9.17)
Streptomycin	37 (18.14)	N/A	13 (11.93)
Pefloxacin	12 (5.88)	10 (20.41)	7 (6.42)
Chloramphenicol	30 (14.71)	7 (14.29)	13 (11.93)
Sulfonamide	25 (12.25)	6 (12.24)	2 (1.83)

Table 5: *Prevalence of antibiotics resistance among animals.*

Antibiotics	Susceptible (%)	Intermediate (%)	Resistance (%)
Rifampicin	15 (13.16)	2 (16.67)	0
Gentamycin	12 (10.53)	N/A	5 (100)
Erythromycin	17 (14.91)	0	0
Ciprofloxacin	12 (10.53)	5 (41.67)	0
Streptomycin	17 (14.91)	N/A	0
Pefloxacin	8 (7.02)	4 (33.33)	0
Chloramphenicol	17 (14.91)	0	0
Sulfonamide	16 (14.04)	1 (8.33)	0

Among the livestock sampled, rate of MRSA isolation was highest in sheep. This agrees with the work of

Gulani et al. (2016) which stated that among livestock and animal handlers, sheep recorded the highest. However, a study in Maiduguri in 2016 disagrees with this study; it stated a lower isolation of MRSA in sheep (4.6%) than other ruminants (Mai-siyama, 2014). In France, relatively high prevalence of MRSA among cattle (44.8%) was recorded, in Tunisia (29%) and Saudi Arabia (21.8%) (Gharsa et al., 2012).

Originally it was thought that MRSA transmission was basically from human to animal, with MRSA colonization and infection occurring through contact between human hands and anterior nares of the animal. There is now increasing evidence that MRSA can be transmitted in both directions; zoonosis and reverse zoonosis that is from animal to human and human to animal respectively. When animals get exposure to and colonized by MRSA, they could become reservoir hosts of this organism to other animals and their human handlers (Weese, 2005). Possible transmission and dissemination of the MRSA isolates could occur through contact which includes proximity through rearing and treating, domestication, nasal dropping during movement within the community and contamination of the meat by infected handlers (Lee, 2003). Livestock associated MRSA can enter a hospital setting through people with nasal colonization or through in-patients who needed treatment for MRSA infection according to Cuny et al. (2015). The Cuny et al. (2015) study confirmed a rise in livestock associated MRSA in hospital setting in Germany from 14% in 2008 to 23% in 2011. An outbreak can be the outcome in nearby communities as well as livestock farm, if not controlled. The implication is the circle of infection and reinfection of MRSA within and outside livestock farms increases the difficulty of treating diseases caused by this methicillin resistant organism in livestock and humans. Therefore, to increase preventive measures of MRSA colonization, the use of masks and hand gloves can be considered knowing that transmission of *S. aureus* occurs primarily through contact from hand to nose.

From Table 2, there is no statistical significant difference between the prevalence of MRSA in male and female students which is consistent with the findings of Rodriguez et al. (2014) and Fan et al. (2016). However, there was statistical association between the prevalence of MRSA in livestock, and students. It was also discovered that there was a strong linear correlation between MRSA in livestock, and students indicating that the MRSA increase in livestock results in an increase in students' getting infected. This is in line with the findings of Ye et al. (2016) that pig workers carried MRSA and MDRSA isolates with multiple markers of livestock-association (tetracycline-resistance, IEC-negative, and CC9). Prospective studies are needed to investigate MRSA transmission between animals and humans and implement preventive measures.

The results of overall antimicrobial resistance (AMR) to other antibiotics showed Gentamycin to nave the highest prevalence of 31.19% for among students and 100% for livestock. A study agreed with a higher frequency of Gentamycin and Erythromycin resistance (Shibabaw et al., 2014). However, it contradicts the work of Garoy et al. (2019) where Gentamycin had 1.2%. Isolation of multiple drug resistance *S. aureus* has over the years been connected with the abuse of antibiotics, contact with livestock, and low standard of infection control, and prevention practices in Nigeria (Aiken et al., 2014). In addition, *S. aureus* isolates from students were resistant to more number of antibiotics than those of livestock. This is in agreement with the work of Castillo et al. (2014) which stated that the reason may be nontherapeutic use of antibiotics in food-animal rearing.

However, susceptibility pattern of *S. aureus* isolates from students indicated that the isolates were highly susceptible to Rifampin (18.63%), Streptomycin (18.14%), Chloramphenical (14.71%), Sulfonamide (12.25%) and Pefloxacin (5.88%). Resistant pattern showed Gentamycin to be highest (31.19%) followed by Erythromycin (20.18%) and the least was Sulfonamide (1.83%). There was more percentage resistance to the antibiotics used than the percentage susceptible. The isolates from animals were very susceptible to the antibiotics used. This study highpoints the importance of regular surveillance of antibiotic susceptibility pattern of *S. aureus* and MRSA to select appropriate therapy.

In considering the result of the study the prevalence of MRSA in livestock and students in the College of Animal Health and Production Technology using chi-squared test of independence and a P-Value of 5% significant level, X^2=7.939, P=0.047 the alternative hypothesis was accepted, meaning there is an association between the prevalence of MRSA in

livestock and students.

The correlation coefficient between prevalence of MRSA in livestock and students is +3 which show a strong positive correlation. The increased in MRSA in livestock also lead to the increased in students MRSA and vice versa.

One of the major limitation to this work is that molecular analysis of samples was not done. This would have enabled confirmation of strains from human sample to be of animal origin or vice versa. Further work is advocated on this.

Conclusions and Recommendations

Veterinary and animal production students are at increased risk of being MRSA reservoirs and could lead to zoonotic transmission to human populace outside the academic environment leading to Community Associated - MRSA (CA-MRSA). Therefore, MRSA colonization should be considered an occupational risk for members of the veterinary, animal health and production team – staff and students inclusive, particularly those in large animal practices. In addition, these professionals should be educated on the risk of MRSA transmission from livestock, and their environment. To add to the robustness of this work, differentiating human from livestock associated MRSA should further be done using molecular markers.

Acknowledgements

We are grateful to the management of Federal College of Animal Health and Production Technology, Vom for approving the collection of samples from students and livestock. Staff members of Microbiology Laboratory of the College are appreciated.

Authors' Contributions

KNA developed the concept, wrote the protocol, supervised the bacteriological assays, carried out the data analysis and drafted the manuscript. JJK collected the samples and participated in carrying out the bacteriological assays. CAL participated in the final proofreading of the manuscript, engaged in bacteriological assays and contributed in the interpretation of the result. KNO participated in the final proofreading of the manuscript, engaged in bacteriological assays and contributed in the interpretation of the result. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflict of interests.

References

Aiken,A.M.,Mutuku,I.M.,Sabat,A.J.,Akkerboom, V., Mwangi, J., Scott, J.A.G., Morpeth, S.C., Friedrich, A.W. and Grundmann, H., 2014. Carriage of *Staphylococcus aureus* in Thika level 5 hospital, Kenya: A cross-sectional study. Antimicrob. Resist. Infect. Control. 3(1): 22. https://doi.org/10.1186/2047-2994-3-22

Aires-de-Sousa, M., 2017. Methicillin-resistant *Staphylococcus aureus* among animals: Current overview. Sci. Direct. Clin. Microbiol. Infect., 23(6): 373-380. https://doi.org/10.1016/j.cmi.2016.11.002

Castillo, N.R, Frisancho, J.A., Rinsky, J.L., Resnick, C., Carroll, K.C., Rule, A.M., Ross, T., You, Y., Price, L.B. and Silbergeld, E.K., 2014. Multidrug-resistant and methicillin-resistant *Staphylococcus aureus* (MRSA) in hog slaughter and processing plant workers and their community in North Carolina (USA). Environ. Hlth. Perspect. 122: 471–477. https://doi.org/10.1289/ehp.1306741

Cheesebrough, M., 2000. Distinct laboratory practical in tropical countries part 2. 1st edition. Cambridge Univ. Press. pp. 136.

CLSI. 2014. Standards for antimicrobial disk susceptibility tests. Approved standard. In *Ninth edition Document M2-A9* Clinical and Laboratory Standards Institute, Wayne, PA.

Cuny, C., Wieler, L.H. and Witte, W., 2015. Livestock-associated MRSA: The Impact on humans. Antibiotics, 4: 521-543. https://doi.org/10.3390/antibiotics4040521

Dilip, N., 2003. Impact of methicillin resistant *Staphylococcus aureus* infection on key health economic outcomes. J. Antimicrobe. Chemother. 3(11): 37-44. https://doi.org/10.1093/jac/dkg250

Fan, Y., Wang, X., Li, L., Yao, Z., Chen, S. and Ye, X., 2016. Potential relationship between phenotypic and molecular characteristics in revealing livestock-associated staphylococcus

aureus in Chinese humans without occupational livestock contact. Front. Microbiol. 7: 1517. https://doi.org/10.3389/fmicb.2016.01517

Fox, J. and Bouchet-Valat, M., 2019. Rcmdr: R Commander. R package version 2.5-3.

Garoy, E.Y., Gebreab, Y.B., Achila, O.O., Tekeste, D.G., Kesete, R., Ghirmay, R., Kiflay, R. and Tesfu, T., 2019. Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence and Antimicrobial sensitivity patter among patients – a multicenter study in Asmara, Eritrea. Can. J. Infect. Dis. Med. Microbiol. Article ID 8321834, 9 pages. https://doi.org/10.1155/2019/8321834

Gharsa, H., Slama, K.B., Lozano, C., Gomez-sanz, E., Sallem, R.B., Go'mez, P., Zarazaga, M., Boudabous, S.A., and Torre, C., 2012. Prevalence, antibiotic resistance virulence traits and genetics lineage of *staphylococcus aureus* in healthy. Vet. Microbiol. 156(3-4): 367-373. https://doi.org/10.1016/j.vetmic.2011.11.009

Grøntvedt, C.A., Elstrøm, P., Stegger, M., Skov, R.L., Andersen, P.S., Larssen, K.W., Urdahl, A.M., Angen, O., Larsen, J., Åmdal, S., Løtvedt, S.M., Sunde, M. and Bjørnholt, J.V., 2016. Methicillin-resistant *Staphylococcus aureus* CC398 in humans and pigs in Norway: A "One health" perspective on introduction and transmission. Oxf. J., Clin. Infect. Dis. 63(11): 1431-1438. https://doi.org/10.1093/cid/ciw552

Gulani, I.A., Geidan, Y.A., Adamu, L., Lawal, J.R., and Abadam, F.A., 2016. Prevalence and phynotipic detection of methicillin resistant *Staphylococcus aureus* between ruminants in Maiduguri, Nigeria. J. Adv. Vet. Anim. Res. 3(2): 152-159. https://doi.org/10.5455/javar.2016.c145

Hema, N., Raj, N.S., Chaithanya, E.D., Chincholi, R., Iswariya, M. and Hema, K.N., 2017. Prevalence of nasal carriers of methicillin-resistant *Staphylococcus aureus* among dental students: An *in vivo* study. J. Oral Maxillofac. Pathol. 21: 356-359. https://doi.org/10.4103/jomfp.JOMFP_212_17

Khalid A. Ali Abdel Rahim and Ahmed Mohamed Ali Mohamed. 2015. Bactericidal and antibiotics synergistic effect of Nanosilver against methicillin-resistant *Staphylococcus aureus*. Jundishapur J. Microbiol. 8(11): 1-6. https://doi.org/10.5812/jjm.25867

Lee, B.Y., Singh, A., David, M.Z., Bartsch, S.M., Slayton, R.B., Huang, S.S., Zimmer, S.M., Potter, M.A., Macal, C.M., Lauderdale, D.S., Miller, L.G. and Daum, R.S., 2013. The economic burden of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Clin. Microbiol. Infect. 19(6): 528–536. https://doi.org/10.1111/j.1469-0691.2012.03914.x

Lee, J.H., 2003. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from food animals and their potential transmission to humans. Appl. Environ. Microbiol. 69: 6489-6494. https://doi.org/10.1128/AEM.69.11.6489-6494.2003

Mai-siyama, I.B., Okon, K.O., Adamu, N.B., Skira, U.M., Ishaka, T.M., Adamu, S.G., and Mohammed, A., 2014. Methicillin resistant *Staphylococcus aureus* (MRSA) colonization rate among ruminant animals slaughtered for human consumption and contact persons in Maiduguri, Nigeria. Afr. J. Microbiol. Res. 8: 2643-2649. https://doi.org/10.5897/AJMR2014.6855

Pantosti, A., Sanchimi, A. and Monaco, M., 2007. Mechanism of antibiotic resistance in *Staphylococcus aureus*. Future Microbiol., 2: 323-324 Public Medicine. https://doi.org/10.2217/17460913.2.3.323

Rodríguez, E.A., Correa, M.M., Ospina, S., Atehortúa, S.L. and Jiménez, J.N., 2014. Differences in epidemiological and molecular characteristics of nasal colonization with *Staphylococcusaureus* (MSSA-MRSA) in children from a university hospital and day care centers 2014; PLoS One, 9: e101417. https://doi.org/10.1371/journal.pone.0101417

Shibabaw, A., Abebe, T. and Mihret, A., 2014. Antimicrobial susceptibility pattern of nasal *Staphylococcus aureus* among Dessie referral hospital health care workers, Dessie, Northeast Ethiopia. Int. J. Infect. Dis. 25: 22–25. https://doi.org/10.1016/j.ijid.2014.03.1386

Voss, A., Loeffen, F., Bakker, J., Klaassen, C. and Wulf, M., 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg. Infect. Dis. 11(12): 1965-1966. https://doi.org/10.3201/eid1112.050428

Weese, J.S., 2005. Methicillin-resistant *Staphylococcus aureus*: an emerging pathogen in small animals. *J. Am. Anim. Hosp. Assoc.* 41: 150-157. https://doi.org/10.5326/0410150

Wulf, M., Nes, A.V., Eikelenboom-Boskamp, A., Vries, J., Melchers, W., Klaassen, C. and Voss, A., 2006. Methicillin-resistant *staphylococcus aureus* on veterinary doctors and students, the Netherlands. Emerg. Infect. Dis. 12(12): 1939-1941. https://doi.org/10.3201/eid1212.060355

Ye, X., Fan, X., Wang, X., Liu, W., Yu, H., Zhou, J., Chen, S. and Ya, O.Z., 2016. Livestock-associated methicillin and multidrug resistant *S. aureus* in humans is associated with occupational pig contact, not pet contact. Sci. pp. 19184. https://doi.org/10.1038/srep19184

Zomer, T.P., Wielders, C.C.H., Veenman, C., Hengeveld, P., Hoek, W., Greeff, S.C., Smit, L.A.M., Heederik, D.J., Yzermans, C.J., Bosch, T., Maassen, C.B.M., and Duijkeren, E., 2017. MRSA in persons not living or working on a farm in a livestock-dense area: prevalence and risk factors. J. Antimicrob. Chemother. 72: 893–899. Accessed from https://academic.oup.com/jac/article-abstract/72/3/893/2691390 on 02 May 2019.