sciendo

DOI: 10.2478/fv-2022-0016

FOLIA VETERINARIA, 66, 2: 37—45, 2022

OCCURRENCE AND ANTIBIOTIC RESISTANCE PROFILES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN LAYER CHICKENS IN KEBBI, NIGERIA

Gaddafi, M. S.[1], Yakubu, Y.[2], Bello, M. B.[3], Bitrus, A. A.[4], Musawa, A. I.[2] Garba, B.[2], Lawal, H.[1], Aliyu, M. A.[1], Barka, S. A.[5], Emeka, A. J.[2]

[1]Ministry of Animal Health, Husbandry and Fisheries, Kebbi State
[2]Department of Veterinary Public Health and Preventive Medicine
[3]Department of Veterinary Microbiology, Faculty of Veterinary Medicine
Usmanu Danfodiyo University, Sokoto State
[4]Department of Veterinary Microbiology and Pathology, Faculty of Veterinary Medicine
University of Jos, PMB 2084 Jos, Plateau
[5]Agricultural Research Council of Nigeria
Nigeria

gaddafimohammedsani@yahoo.com.

ABSTRACT

Antimicrobial resistance (AMR) is a global health threat, and antimicrobial use in animal production for growth enhancement or prophylaxis contributes to the development of AMR. A cross-sectional study was conducted to investigate the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in poultry and farm attendants in Kebbi, North-Western Nigeria. A total of 207 cloacal and nasal swabs were randomly collected from four farms comprising 50 samples from each poultry farm and only 7 samples from farm attendants. The samples were analysed using routine bacteriological culture and identification techniques. Presumptive MRSA isolates were confirmed by PCR assay and AMR profiles of the isolates were evaluated using the Kirby-Bauer disk diffusion method. Of the 207 samples examined, 37.5 % (75/200) of layer birds tested positive for MRSA and 71.4 % (5/7) of farm attendants were MRSA positive. All the isolates were susceptible to vancomycin, with an AMR index > 0.3. The findings of this study indicated colonization of layer chickens and humans by multidrug resistant MRSA, thus highlighting the potential role of poultry sources of transmission of multidrug-resistant MRSA strains to humans and vice versa.

Key words: antibiotics; chickens; Kebbi; Nigeria

INTRODUCTION

Antimicrobial resistance (AMR) is a worldwide health concern [35]. AMR is considered as one of the most serious public health threats of the century [9, 25, 36]. Over the years, research has shown a considerable empirical evidence highlighting the contribution of antimicrobial use (AMU) to the overall burden of AMR emergence [4, 19]. A major contributing factor to this emergence is the misuse of antimicrobials in livestock production. The magnitude of usage is expected to increase considerably in the future due to increased livestock farming activities [31]. Most of the knowledge and assumptions on the prevalence and evolution of AMR in animal production systems relate to

organisms that often are considered commensals in poultry such as *Escherichia coli* [23], and *Staphylococcus aureus* [25].

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been detected in several species of animals [34]. Livestock can act as a reservoir and source for the emergence of novel MRSA clones in humans [14]. Livestock acquired methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in farmers constitutes a major threat to public health and the health care system [5, 34]. MRSA prevalence in humans has been reported to be strongly associated with the prevalence in animals and intensity of contact with animals positive for methicillin-resistant *Staphylococcus aureus* [24]. LA-MRSA has been reported to cause infections amongst farm workers and their relatives [14].

Researchers have implicated poultry as reservoirs for AMR bacteria that may spread to humans, with poultry farming widely believed to be a major risk factor for AMR in humans [24]. However, quantitative evidence describing the role of poultry in the emergence and transmission of AMR bacteria to human populations is lacking [21], particularly in Kebbi State, Nigeria. In the absence of routine surveillance of AMR in Kebbi State, understanding the prevalence of AMR is key to developing effective strategies targeted towards reduction in the emergence and spread of such resistance genes in the future.

This study investigates the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and its multi-drug resistant index in poultry farms and their attendants in the Kebbi State, Nigeria.

MATERIALS AND METHODS

Ethical approval

All experiments were done in accordance with the guidelines to the use and care of animals of the Research Ethics Committee of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto (UDUS/FAREC/03/2019).

Study area

Kebbi is in North-Western Nigeria and lies between latitude 12.45 0N and longitude 4.2 0E. Kebbi State has an agriculturally viable environment since it is endowed with high soil fertility, vast farmlands and economically viable rivers sheltered by a fine tropical climate. Agriculture has remained the major source of revenue and indeed, the backbone of the economy of the state. Major food crops in the area are: millet, guinea-corn, maize, cassava, potatoes, rice, beans, onions, and vegetables.

Sample size determination

A total of 200 cloacal swab samples were randomly collected from four different farms within the State. The sample size was determined using an estimated prevalence of 13.7% as reported by Kwoji et al. [17] in the formula described by Thrusfield [30]:

$$N = \frac{t^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

where

t^2 = Standard deviation $(1.96)^2$

P_{exp} = Expected MRSA prevalence by Kwoji [17] 13.7% i.e. 0.137

d^2 = $(0.05)^2$

$$N = \frac{1.96^2 \times 0.137\,(1 - 0.137)}{0.025} = 180.16$$

Study design and sample collection

A cross-sectional study was conducted between April and June 2019. Cloacal swabs were collected from each chicken. A total of two hundred laying birds (fifty samples from each farm) were randomly sampled from four poultry farms in the study area. Also, nasal swabs were taken from apparently healthy farm attendants (whom have not used antibiotics in the last three months) after seeking their consent. Only seven (7) farm attendants volunteered to partake in the study (Farm A: 1, Farm B: 3, Farm C: 1, Farm D: 2).

Bacteriological culture and isolation

Upon arrival to the laboratory (central research laboratory, Usmanu Danfodiyo University Sokoto), cloacal swabs from poultry were pre-enriched in peptone water for 24 h at 37°C before inoculation on blood agar containing 5% horse blood and incubated aerobically at 37°C for 24 h. Presumptive colonies that characteristically appeared like Staphylococcus spp. were preliminarily identified using colony morphology and Gram staining for cellular

morphology. Colonies that appeared relatively small, circular, convex, smooth, and grayish to white were picked and streaked on a freshly prepared Mannitol Salt agar (Oxoid, Basingstoke, UK) and incubated aerobically at 37 °C for 24 h. Two to three presumptive colonies of the Staphylococcus species that appeared small, smooth, golden, shiny, convex, and with golden yellow zones were picked and sub-cultured in nutrient agar (Oxoid) slant and incubated at 37°C for 18—24 h. Staphylococcus isolates were further confirmed biochemically using catalase and coagulase tests as described by Ochei, Kohlhatkar [26] and Cheesbrough [6].

Phenotypic characterization of MRSA

Overnight fresh cultures of presumptive *S. aureus* were inoculated onto a freshly prepared Oxacillin Resistance Screening Agar Base using appropriate supplement (ORSAB; Oxoid, Basingstoke, UK) to determine phenotypic methicillin resistance. Presumptive colonies on ORSAB that appeared deep blue in color on a colorless background were phenotypically considered as MRSA. Media preparation and interpretation of colonies were carried out as described in the manufacturer's guide.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing for 7 antibiotics; erythromycin (E 15 µg), oxytetracycline (OXT 30 µg), neomycin (N 5 µg), penicillin (P 30 µg), sulfonamide (SUL 23.75 µg), gentamicin (CN 10 µg) and vancomycin (VAN 30 µg) that are commonly used in veterinary and human medicine in Kebbi State was studied using the disc diffusion method (Oxoid Ltd, Basingstoke, UK). Antibiotic discs were dispensed onto bacteria-containing agar plates and incubated for a period of 18 hours at 35 °C, according to standard protocols. Zones of inhibition were recorded and interpreted according to the recommendations of the Clinical and Laboratory Standards Institute CLSI [8].

Multiple antimicrobial resistance index (MARI) was also determined by dividing the number of antibiotics each isolate is resistant to by the total number of antibiotics included in the panel.

Genotypic characterization of MRSA

Genomic DNA extraction

Genomic DNA was extracted using the traditional boiling method as described by Chen et al. [7] with slight modification. Briefly, a loop-full suspension of overnight grown cultures on nutrient agar plate was transferred into a 1.5 ml micro-centrifuge tube containing 100 µl of sterile distilled water. The suspension was first incubated at room temperature for 5 min, and then heat treated to 96 °C for 10 min. This was then followed by centrifugation at 12,000 x g for 5 min. The supernatant (containing the DNA) was then collected in a new 1.5 ml tube using a micropipette and kept at 4 °C until used.

PCR detection of *mecA* gene

DNA templates of all the ORSAB positive *S. aureus* isolates were subjected to PCR assay for the detection of the 163 bp fragment of the *mecA* gene using the following primers; *MecA*1 5'-AAAATCGATGGTAAAGTTGGC-3'(forward), *MecA*2 5'AGTTCTGCAGTACCGGATTTGC-3'(reverse) as described by Mehrotra et al. [20]. The PCR assay was performed in a 25 µl reaction mixture containing 3 µl of nuclease free water, 5 µl (0.5 µg) of the DNA template, 1 µl (0.2 µM) of each forward and reverse primer, 2.5 µl of coral red dye and 12.5 µl (100 µM) of the master mix (Qiagen). The amplification protocol comprised of 35 cycles of amplification with an initial denaturation of 94 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, extension at 72 °C for 1 min and then final extension at 72 °C for 7 min. The PCR products were visualized after electrophoresis for 45 min at 90 volts in a 1% agarose gel. The amplicons were then viewed under a UV trans-illuminator (Biorad Gel Doc XR System w/ Universal Hood II).

Data analysis

The results obtained were presented in tables. Chi-squared statistics were performed using Invivostat v 4.1 to determine possible association between MRSA infection and farms sampled.

RESULTS

***S. aureus* and MRSA in poultry and farm attendants**

Of the 200 poultry cloacal swabs examined 72.0% (144/200) were positive for *Staphylococcus aureus* based on culture, biochemical and molecular characterization. Farm B had the highest prevalence with 42/50 (84%), followed by Farm A with 39/50 (78%). Farms D and C had

Table 1. Distribution of *Staphylococcus aureus* in poultry farms in Kebbi state

Grouping factor	No. of samples	+ve *S. aureus*	χ2	Df	P-value
Farm A	50	39 (78%)			
Farm B	50	42 (84%)	9.72	3	0.02
Farm C	50	29 (58%)			
Farm D	50	34 (68%)			

Df—degree of freedom

Table 2. Distribution of MRSA in Poultry farms in Kebbi State

Grouping factor	No. of samples	+ve MRSA	χ2	Df	P-value
Farm A	50	25 (50%)	7.91	3	0.04
Farm B	50	21 (42%)			
Farm C	50	17 (34%)			
Farm D	50	12 (24%)			

Df—degree of freedom

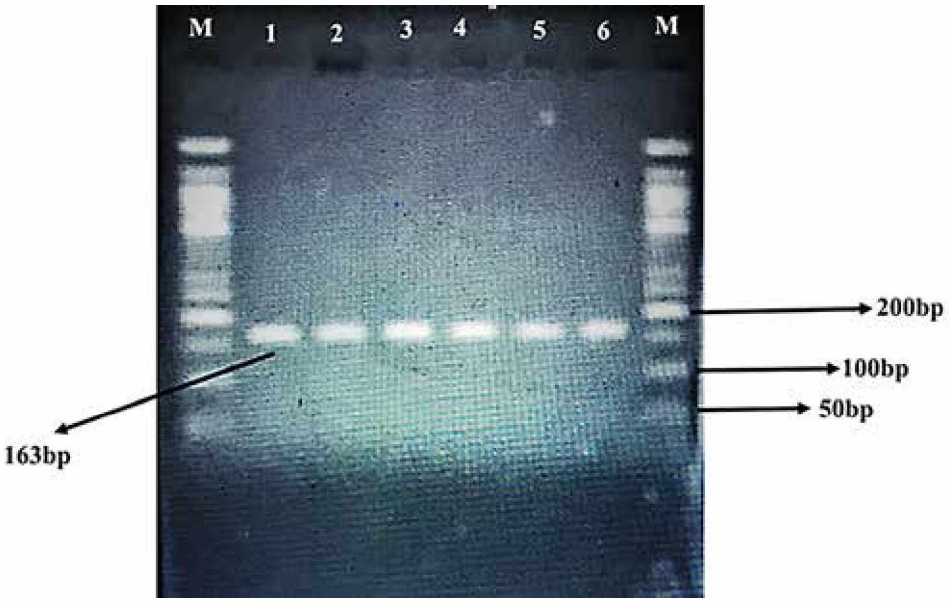

Fig. 1. Gel image of 163bp gene fragment of the *mecA* gene using 50 bp molecular weight DNA ladder

34/50 (68%) and 29/50 (58%) frequencies, respectively. There was a statistically significant association (P=0.02) between the recovery rate of *S. aureus* and Farms with farm B more likely to have *S. aureus* colonization than the other farms (Table 1).

In this study, phenotypic detection of MRSA on ORS-AB showed that 37.5% (75/200) of cloacal swabs were positive. Farm A had the highest frequency of 25/50 (50%), followed by Farm B with a frequency of 21/50 (42%), Farms C and D had 17/50 (34%) and 12/50 (24%)

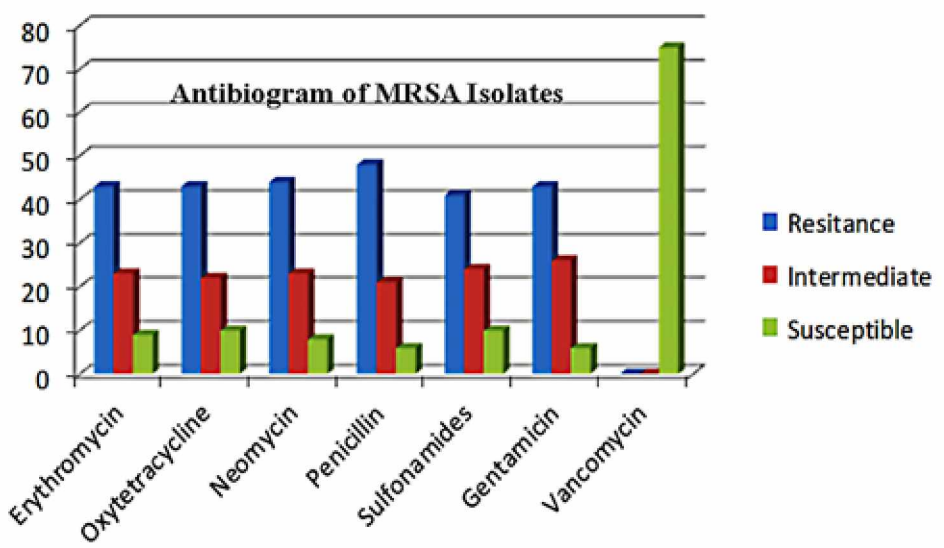

Fig. 2. Radar chat of antibiotic sensitivity profile of MRSA isolates (n = 75) from poultry farm in Kebbi State

Table 3. Antibiotic resistant pattern of MRSA isolates from poultry farms in Kebbi State

Antibiotic resistant pattern	Number of isolates	Multiple antibiotic resistance index
ERY-OXY-NEO-SUL-GEN	1	0.71
OXY-NEO-PEN-SUL-GEN	1	0.71
ERY-OXY-NEO-PEN-GEN	1	0.71
ERY-PEN-SUL-GEN	2	0.60
OXY-NEO-PEN-SUL	2	0.60
ERY-OXY-NEO-PEN-GEN	2	0.71
ERY-OXY-NEO-PEN	5	0.60
ERY-OXY-NEO-PEN-SUL	13	0.71
ERY-OXY-NEO-PEN-SUL-GEN	17	0.86

frequencies, respectively. There was a statistically significant association (P=0.04) between the recovery rate of MRSA and Farms with farm A more likely to have MRSA colonization than the other farms (Table 2). The seven farm attendants sampled and examined, showed 5/7 (71.4%) colonization rate for both *S. aureus* and MRSA. PCR amplification of all the eighty (80) positive samples on ORSAB for the detection of *mecA* gene showed 100% positivity (Figure 1). Hence, the overall molecular detection rate of MRSA from poultry farms studied was 38.6% (80/207).

Antimicrobial resistance profiles of *S. aureus* and MRSA from poultry and farm attendants

Antimicrobial susceptibility tests revealed that the ORSAB positive isolates exhibited varying level of resistance against the antimicrobials tested. Out of the 75 MRSA isolates recovered from poultry farms, 43 (57.3%) of them were resistant to erythromycin, oxytetracycline and gentamycin, 44 (58.7%) were resistant to neomycin, 41 (54.7%) and 48 (64%) were resistant to sulfonamides and penicillin respectively. All isolates were susceptible to vancomycin (Figure 2). A total of 17 isolates were found to show multidrug resistance (MDR) with ERY-OXY-NEO-PEN-SUL-GEN being the most common pattern and the multiple antibiotic resistance (MAR) index is > 0.2 (Table 3).

DISCUSSION

The emergence of MRSA does not only pose significant public health threat challenge but also threatens animal health as an emerging veterinary pathogen of great importance throughout the world. Over time, there is a steady increase in the prevalence of MRSA colonization in healthy food producing animals [10, 29].

In this study, the overall occurrence rate of MRSA from poultry farms was 38.6% with the following rates among different farms (Farm A: 50%, Farm B: 42%, Farm C: 34% and Farm D: 24%) and 2.4% in farm attendants.

The isolation rate in our study was much higher than the 8.82%, 6.3% and 7.9% recorded by Kwoji et al. [17], Bale et al. [2] and Musawa et al. [22], respectively. These variations could be attributed to difference in the sensitivity of the detection method, sample size, sample source and the indiscriminate use of antimicrobials in animal production [13]. It is noteworthy that Musawa et al. [22] conducted their research on poultry carcass rinse while Kwoji et al. [17] and Bale et al. [2] only used phenotypic characteristics to determine MRSA in their study. Contrarily, all *S. aureus* isolates from apparently healthy animals from Tunisia and China were methicillin-susceptible [15, 38]. The absence of MRSA in these studies could be attributed to the good management practices in the study areas as administration of antibiotics is regulated in animal farming. The occurrence of MRSA in poultry in this study however could be due to indiscriminate use of antibiotics in poultry production by farmers without prescription by a veterinarian [19]. MRSA is probably the best example of a prevalent and important multidrug-resistant bacterium that has successfully transitioned from an almost exclusively nosocomial setting to being widespread in the community [33].

Most of the MRSA isolates in this study were multidrug-resistant strains (exhibiting resistance to six out of seven antibiotics exposed to), a result of which agreed with the report of Gaddafi et al. [13] in healthy pigs in Kebbi State. The result showed a high resistance to penicillin, oxytetracycline, neomycin, erythromycin, gentamicin, and sulfonamides. Reports from Anueyiagu and Isiyaku [1], Jahan et al. [16] and Gaddafi et al. [12] in agreement showed a remarkably high MRSA resistance to penicillin because it is the drug of choice in *S. aureus* infection. High resistance to oxytetracycline noticed in this study could be attributed to the assertion by Olatoye [27] who opinioned that tetracycline is commonly used as a feed additive and for prophylaxis in animal production in Nigeria. Similarly, Usman et al. [32] opinioned that resistance to tetracycline is a major problem in veterinary practices in Nigeria due to availability and affordability of the medication. High resistance to erythromycin and gentamicin observed in this study could possibly reflect its frequent use in livestock in the study area. In agreement to this, Anueyiagu and Isiyaku [1], reported significant levels of resistance to erythromycin and gentamicin in Nigeria. Results of MRSA susceptibility testing from this study showed 100% susceptibility to vancomycin. This finding is unsurprising because vancomycin is rarely used in the treatment of diseases in livestock in the study area [13].

Forty-four MRSA strains isolated from this study showed multidrug resistance. The isolates were resistant to a combination of the four, five and six antibiotics tested. Bitrus et al. [3] explained that multi-drug resistance in *S. aureus* may be partly attributed to the acquisition of resistance determinants domiciled in mobile genetic elements. Lay et al. [18] also opined that the determinants of multi-drug resistance are capable of being disseminated in a region or between regions because of antibiotic selective pressure in either livestock or humans. All methicillin resistant *S. aureus* examined in this study had an MAR index of 0.6 and above. The MAR index gives an indirect indication of the probable source of an organism. An organism originates from an environment with high levels of antibiotic use when its MAR index is greater than 0.2 [11]. The findings of the current study underscore the circulation of multidrug-resistant MRSA strains among apparently healthy poultry farms and their attendants and accordingly, poultry may be considered as a reservoir for such multidrug-resistant strains which may easily pass to humans through direct contact resulting in horizontal transmission between humans, with great public health consequences. More so, in Nigeria, the indiscriminate use of antibiotics in livestock production could have also contributed immensely to the patterns and index of antibiotic resistance recorded in this study.

To date, the correlation between MRSA phenotype and genotype must be considered from the surrogate test for oxacillin and *mecA* gene existence [13]. In this study, all eighty MRSA strains examined carried the *mecA* gene, indicating that the phenotypic resistance exhibited by the strains on ORSAB was due to the possession of the gene. This finding agrees with the report of Gaddafi et al. [13] who also detected *mecA* gene in all the forty-four MRSA strains isolated from pig farms and their attendants in Zuru. Contrarily, our findings differed from the report of Musawa et al. [22] and Yakubu et al. [37] who detected the *mecA* gene in only fifteen out of thirty-seven and twelve out of twenty-two MRSA isolated from poultry carcass rinse and some retailed animal products in Sokoto, respectively. Phenotypic expression of resistance to methicillin in MRSA varies and each strain has a characteristic

profile of the proportion of bacterial cells that grow at specific concentrations of methicillin [28].

CONCLUSIONS

The antibiotic susceptibility profiles of the MRSA strains isolated from poultry showed significant levels of resistance to penicillin, erythromycin, gentamicin, and oxytetracycline. This finding is of great public health concern because these antibiotics are commonly used in Nigeria either therapeutically in human and veterinary practices or as growth promoters and for prophylaxis in poultry production. This also highlighted the roles of layer chickens and possibly eggs as potential sources of infection to humans.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the staff of Central Research Laboratory, Faculty of Veterinary Medicine, UDUDS and the staff of Department of Veterinary Services, Ministry of Animal Health, Husbandry and Fisheries, Kebbi State for provision of technical support. This research work was self-funded.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with regards to the publication of this manuscript.

REFERENCES

1. **Anueyiagu, K. N., Isiyaku, A. W., 2015:** Isolation and identification of *Staphylococcus aureus* from bovine milk and its antibiotic susceptibility. *Int. J. Livest. Product.*, 6, 6, 74—77. DOI: 10.5897/IJLP2015.0248.
2. **Bale, M. I., Babatunde, S. K., Adedayo, M. R., Ajiboye, A. E., Ajao, A. T., 2019:** Characterization of methicillin-resistant *Staphylococcus aureus* isolates from apparently healthy individuals in Malete, Kwara state, Nigeria. African *J. Clin. Exper. Microbiol.*, 20, 1, 17—24. DOI: 10.4314/ajcem.v20i1.3.
3. **Bitrus, A. A., Zunita, Z., Goni, M. D., Mshelia, I. T., 2018:** Dissemination of resistance and virulence determinants in methicillin-resistant *Staphylococcus aureus* during colonization and disease: A review. *Adv. Anim. Vet. Sci.*, 6, 1, 44—54. DOI: 10.17582/journal.aavs/2018/6.1.44.54.
4. **Bortolaia, V., Espinosa-Gongora, C. and Guardabassi, L., 2016:** Human health risks associated with antimicrobial-resistant enterococci and *Staphylococcus aureus* on poultry meat. *Clin. Microbiol. Infect.*, 22,130—40. DOI: 10.1016/j.cmi.2015.12.003.
5. **Broens, E. M., Graat, E. A., Van der Wolf, P. J., Giessen, A., De Jong, M., 2011:** Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. *Prev. Vet. Med.*, 102, 1, 41—49. DOI: 10.1016/j.prevetmed.2011.06.005.
6. **Cheesbrough, M., 2000:** *District Laboratory Practice in Tropical Countries*. Part II. Cambridge University Press, Cambridge, UK. 65, 38—39.
7. **Chen, L., Mediavilla, J. R., Oliveira, D. C., Willey, B. M., De Lencastre, H., Kreiswirth, B. N., 2009:** Multiplex real-time PCR for rapid staphylococcal cassette chromosome mec typing. *J. Clin. Microbiol.*, 47, 11, 3692—3706. DOI: 10.1128/JCM.00766-09.
8. **Clinical and Laboratory Standards Institute 2018:** *Performance Standards for Antimicrobial Susceptibility Testing*. 28th edn., CLSI supplement M100. Wayne, PA. 130—134
9. **Davies, S. C., Fowler, T., Watson, J., Livermore, D. M., Walker, D., 2013:** Annual report of the Chief Medical Officer: infection and the rise of antimicrobial resistance. *Lancet Infect. Dis.*, 381, 9878, 1606—1609. DOI: 10.1016/S0140-6736(13)60604-2.
10. **El-Deeb, W., Fayez, M., Elmoslemany, A., Kandeel, M., Zidan, K., 2018:** Methicillin resistant *Staphylococcus aureus* among goat farms in Eastern province, Saudi Arabia: Prevalence and risk factors. *Prev. Vet. Med.*, 156, 84—90. DOI: 10.1016/j.prevetmed.2018.05.005.
11. **Furtula, V., Jackson, C. R., Farrell, E. G., Barrett, J. B., Hiott, L. M., Chambers, P. A., 2013:** Antimicrobial resistance in Enterococcus spp. isolated from environmental samples in an area of intensive poultry production. *Int. J. Environ. Res. Pub. Health*, 10, 3, 1020—1036. DOI: 10.3390/ijerph10031020.
12. **Gaddafi, M. S., Yakubu, Y., Garba, B., Bello, M. B., Musawa, I. A., Lawal, H., 2020:** Occurence and antimicrobial resistant patterns of methicillin resistant *Staphylococcus au-*

reus (MRSA) among practicing veterinarians in Kebbi State, Nigeria. *Folia Vet.*, 64, 55—62. DOI: 10.2478/fv-2020-0038.

13. **Gaddafi, M. S., Yakubu, Y., Junaidu, A. U., Bello, M. B., Garba, B., Bitrus, A. A., et al., 2021:** Nasal colonization of pigs and farm attendants by *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in Kebbi, Northwestern Nigeria. *Thai J. Vet. Med.*, 51, 1, 119—124. DOI: 10.14456/tjvm.2021.16.
14. **García-Álvarez, L., Holden, M. T., Lindsay, H., Webb, C. R., Brown, D. F., Curran, M. D., et al., 2011:** Methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect. Dis.*, 11, 8, 595—603. DOI: 10.1016/S1473-3099(11)70126-8.
15. **Gharsa, H., Slama, K. B., Gómez-Sanz, E., Lozano, C., Zarazaga, M., Messadi, L., et al., 2015:** Molecular characterization of *Staphylococcus aureus* from nasal samples of healthy farm animals and pets in Tunisia. *Vector-Borne Zoonot. Dis.*, 15, 2, 109—115. DOI: 10.1089/vbz.2014.1655.
16. **Jahan, M., Rahman, M., Parvej, M. S., Chowdhury, S. M. Z. H., Haque, M. E., Talukder, M. A. K., et al., 2015:** Isolation and characterization of *Staphylococcus aureus* from raw cow milk in Bangladesh. *J. Adv. Vet. Anim. Res.*, 2, 1, 49—55. DOI: 10.5455/javar.2015.b47.
17. **Kwoji, I. D., Tambuwal, F. M., Abubakar, M. B., Yakubu., Y., Bitrus, A. A., Jauro, S., 2017:** Occurrence of methicillin resistant *Staphylococcus aureus* in chickens and farm personnel in Sokoto, Northwestern Nigeria. *J. Adv. Vet. Anim. Res.*, 4, 3, 255—260. DOI: 10.5455/javar.2017.d220.
18. **Lay, K. K., Torio, H. E., Bitrus, A. A., Mala, W., Nuananong, S., Chuanchuen, R., 2020:** Multidrug resistant *Escherichia coli* harboring extended-spectrum β-lactamase-encoding genes isolated from clinically-healthy pigs. *Thai J. Vet. Med.*, 51, 2, 303—310. DOI: 10.14456/tjvm.2021.38.
19. **Marshall, B. M., Levy, S. B., 2011:** Food animals and antimicrobials: Impacts on human health. *Clin. Microbiol. Rev.*, 24, 718—33. DOI:10.1128/CMR.00002-11.
20. **Mehrotra, M., Wang, G., Johnson, W. M., 2000:** Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1 and methicillin resistance. *J. Clin. Microbiol.*, 38, 3, 1032—1035. DOI: 10.1128/JCM.38.3.1032-1035.2000.
21. **Muloi, D., Kiiru, J., Ward, M. J., Hassell, J. M., Bettridge, J. M., Robinson, T. P., et al., 2019:** Epidemiology of antimicrobial-resistant *Escherichia coli* carriage in sympatric humans and livestock in a rapidly urbanizing city. *Int. J. Antimicrob. Agents*, 54, 5, 531—537. DOI: 10.1016/j.ijantimicag.2019.08.014.
22. **Musawa, I. A., Yakubu, Y., Garba, B., Ballah, F. M., Jibril, H. A., Bello, A. S., et al., 2020:** Dressed chicken as potential vehicle for spread of methicillin-resistant *Staphylococcus aureus* in Sokoto, Nigeria. *Future Sci. OA*, 6, 10, 619. DOI: 10.2144/fsoa-2020-0066.
23. **Nhung, N. T., Chansiripornchai, N., Carrique-Mas, J. J., 2017:** Antimicrobial resistance in bacterial poultry pathogens: A review. *Front. Vet. Sci.*, 4, 126. DOI: 10.3389/fvets.2017.00126.
24. **O'Neill, J., 2015:** *Agriculture and the Environment: Reducing Unnecessary Use and Waste. The Review on Antimicrobial Resistance*. Wellcome Trust and HM Government, London, UK. 1—44.
25. **O'Neill, J., 2016:** *Tackling Drug-resistant Infections Globally: Final Report and Recommendations*. Wellcome Trust and HM Government, London, UK. 1—84
26. **Ochei, J. O., Kolhatkar, A. A., 2000:** *Medical Laboratory Science: Theory and Practice*. McGraw Hill Education. 648—657.
27. **Olatoye, I. O., 2010:** The incidence and antibiotics susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Nigeria. *Afric. J. Biotechnol.*, 9, 8, 1196—1199.
28. **Plata, K. B., Riosa, S., Singh, C. R., Rosato, R. R., Rosato, A. E., 2013:** Targeting of PBP1 by β-lactams determines recA/SOS response activation in heterogeneous MRSA clinical strains. *PLOS ONE*, 8, 4, 61083. DOI: 10.1371/journal.pone.0061083.
29. **Rahman, M. M., Amin, K. B., Rahman, S. M. M., Khair, A., Rahman, M., Hossain, A., et al., 2018:** Investigation of methicillin-resistant *Staphylococcus aureus* among clinical isolates from humans and animals by culture methods and multiplex PCR. *BMC Vet. Res.*, 14, 1, 1—6. DOI: 10.1186/s12917-018-16111-0.
30. **Thursfield, M., 2005:** *Veterinary Epidemiology*. 3rd edn., Blackwell science, Ltd., London, UK. 228—246.
31. **Tiseo, K., Huber, L., Gilbert, M., Robinson, T. P., Van Boeckel, T. P., 2020:** Global trends in antimicrobial use in food animals from 2017 to 2030. *Antibiotics*, 9, 12, 918. DOI: 10.3390/antibiotics9120918.
32. **Usman, R. Z., Mustapha, B. M., Mohammed, F. I., 2016:** Isolation and identification of methicillin resistant *Staphylococcus aureus* (MRSA) from traditionally fermented milk "nono" and yoghurt in Kaduna Metropolis, Nigeria. *Food Sci. Qual. Manag.*, 55, 42—50.

33. **Van Duin, D., Paterson, D. L., 2020:** Multidrug-resistant bacteria in the community: Trends and lessons learned. *Infect. Dis. Clin.*, 30, 2, 377—390. DOI: 10.1016/j.idc.2020.08.002.
34. **Waters, A. E., Contente-Cuomo, T., Buchhagen, J., Liu, C. M., Watson, L., Pearce, K., et al., 2011:** Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. *Clin. Infect. Dis.*, 52, 10, 1227—1230. DOI: 10.1093/cid/cir181.
35. **World Health Organization. Antimicrobial Resistance: Global Report on Surveillance, 2014:** Available from: http://www.who.int/drugresistance/documents/surveillance report/en/.
36. **World Health Organization. Global action plan on antimicrobial resistance, 2015:** *WHO*, Geneva, 45 pp.
37. **Yusuf, Y., Aliyu, M. I., Bello, S. A., Fatima, B., Abdullahi, A., Aliyu, M. D., 2020:** Occurrence of methicillin resistant *Staphylococcus aureus* (MRSA) in some retailed animal products in Sokoto, Nigeria. *Alexandria J. Vet. Sci.*, 64, 1. DOI: 10.5455/ajvs.44682.
38. **Zhou, Z., Zhang, M., Li, H., Yang, H., Li, X., Song, X., et al., 2017:** Prevalence and molecular characterization of *Staphylococcus aureus* isolated from goats in Chongqing, China. *BMC Vet. Res.*, 13, 1, 1—8. DOI: 10.1186/s12917-017-1272-4.

Received January 29, 2022
Accepted May 6, 2022