Resistance Modulation of *Staphylococcus aureus* Isolates of Dairy Cattle through Metallic Oxide Nanoparticles

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**ABSTRACT**

The rise in prevalence and drug resistance in *Staphylococcus aureus* of dairy animals require an alternative to antibiotics. Nanoparticles particularly metallic nanoparticles are a wise approach to modulating drug resistance. The current study thus focuses on the replacement of antibiotics with ZnO, MgO, and Fe2O3 nanoparticles. To achieve this goal, 200 cattle milk samples were collected aseptically and screened for subclinical mastitis using the purposive sampling technique. Following Bergey’s manual of determinative bacteriology, *S.aureus* was identified and antibiotic susceptibility was tested using clinical laboratory and standard institute guidelines. On the other hand, resistant strains were put to antibacterial testing assay against metallic nanoparticles both by well diffusion and broth microdilution method. To analyze the data, both probability and non-probability statistical tools were applied using SPSS version 22 of statistical software at 5% probability. The current study showed 24.56% of *S. aureus* positive from commercial dairy while the resistance of these isolates against gentamicin, enrofloxacin, levofloxacin, and vancomycin was found to be 50, 40, 30, 30%, respectively. On the other hand, a disc diffusion assay was concluded with 24.525±0.806 mm (ZnO) followed by 16.475±0.950mm (MgO) and 13.150±1.392mm (Fe2O3). MgO stood first to contribute lowest Minimum inhibitory concentration (MIC) (1.302±0.564 mg/mL) followed by Fe2O3 (2.930±1.691 mg/mL) and ZnO (3.906±0.000 mg/mL). MIC of ZnO was recorded to be 18.23±11.93 mg/mL, 15.63±0.0 mg/mL, 13.02±4.5 mg/mL, 7.81±6.77 mg/mL, 5.21±2.26 mg/mL, and (3.906±0.000 mg/mL) at 04, 08, 12, 16, 20 and 24th h of incubation. A similar trend was followed by other nanoparticles at different hours of incubation. The study thus concludes a rise in resistant strains of *S. aureus* in bovine milk while metallic nanoparticles as effective alternatives.

**INTRODUCTION**

Microorganisms such as *Staphylococcus aureus* have a symbiotic relationship with certain animals and can colonize in them various places (Shoaib et al., 2020). Bovine subclinical mastitis disease (BSM) is common around the world, being one of the diseases causing the greatest losses to the dairy industry (Gomes et al., 2016). Nonetheless, *S. aureus* is the most common cause of subclinical mastitis in cattle, goats, and bovine in Pakistan...
(Javed et al., 2021) and around the globe. Milk output and quality have decreased in the cattle industry because of mastitis caused by S. aureus. S. aureus has become a multidrug-resistant bacterium that equally affects other than udder of the animals (Sarwar et al., 2021) reflecting potential contagiousness for a wider range of infections (Tong et al., 2015).

Methicillin-resistant S. aureus (MRSA), often known as resistant staph or “superbug,” is one of the most common strains of S. aureus responsible for human infections in hospitals and the community. The World Health Organization has designated MRSA as a high-priority microbe for future investigation and treatment (Tacconelli et al., 2018). S. aureus’s ability to parasitize intracellularly and formation of biofilms shield them from host immune responses and antibiotic effects, which pose significant therapeutic challenges. S. aureus is able to survive and multiply in cells by inhibiting the combination of phagosome and lysosome, subverting autophagy, and other methods (Foster et al., 2014). This bacterium causes considerable economic losses, such as a significant drop in milk production, reproductive difficulties, expenditures associated with the culling of animals, higher veterinary medicine costs, and substituting contaminated milk. Antibiotics are frequently used to treat Staphylococcal infections while resistance has become global issue. Nanoparticles’ antibacterial action is influenced by their stability in infected cells and their ability to reach the target site predictably.

The nanoparticles contain phagocytosis (zipper-like and trigger-like) and no phagocytosis transport pathways into cells (Geiser, 2010). There have also been promising results with inorganic nanoparticles applied to treating S. aureus infections. Nanomedicines have recently been touted as a viable solution to the MRSA problem (Kalhapure et al., 2015). Metallic nanoparticles have shown strong antimicrobial properties against multiple bacteria species in multiple studies (Dizaj et al., 2014). Nanoparticles of zinc oxide had bactericidal effects against gram-positive and gram-negative bacteria as well as spores that are resistant to high temperatures and high pressures. As reported in the studies, MgO nanoparticles cause the disruption of cellular membranes, which causes leakage of cellular contents and leads to the death of the bacterial cells (Jin and He, 2011).

The current study was purposed to check the prevalence of S. aureus in milk, antibiogram of MRSA against a wider range of antibiotics, and to evaluate the antibacterial activity of metallic nanoparticles against S. aureus.

### MATERIALS AND METHODS

**Sample collection site**

The present research was focused on the region of districts Khanewal and Lodhran of South Punjab, Pakistan because it is an emerging commercial dairy setup. A total of n=200 cattle milk samples using the purposive sampling method (Thrusfield, 2018) were collected from commercial farms. Each milk sample was checked for subclinical mastitis using the Surf Field Mastitis Test (Muhammad et al., 2010) while positive samples were moved to the Cholistan University of Veterinary and Animal Sciences, Bahawalpur.

**Isolation of S. aureus and MRSA**

Milk samples were centrifuged at 6000g/5 minutes, and sediment was incubated for 24 h at 37°C in sterile nutrient broth. The sediment from the incubated nutrient broth was centrifuged again and swabbed on blood agar aseptically. The pinpoint round colonies were picked up and streaked on differential medium, mannitol salt agar, after 24 h of incubation at 37°C. Following a 24 h incubation period at 37°C, the round colonies on yellow-colored media were selected for gram staining and other biochemical assays (Bergey and Holt, 1994).

All S. aureus isolates were phenotypically identified for MRSA using the oxacillin disc diffusion technique (CLSI, 2018). S. aureus growth was distributed at 10⁸ CFU/mL on Muller Hinton agar. To discover MRSA, a 10 µg oxacillin disc was put on the surface of the zone of inhibition, the diameter of which was measured and compared to standards supplied by the Clinical and Laboratory Standard Institute.

**Antibiogram of MRSA**

Antibiotic susceptibility of MRSA was determined using linezolid, enrofloxacin, chloramphenicol, gentamicin, ciprofloxacin, levofloxacin, fusidic acid, septran, and vancomycin. In a nutshell, new MRSA growth was distributed aseptically at 10⁸ CFU/mL on Muller Hinton agar. Selected antibiotics were put on agar aseptically, stored at 37°C for 18-24 h, and the diameter of inhibitory zones was measured. Isolates were classified as resistant, intermediate, or sensitive using standards from the Clinical Laboratory and Standard Institute (CLSI, 2018).

**Preparation of nanoparticles**

Metallic nanoparticles were obtained from the Department of Chemistry, University of Agriculture Faisalabad. For this purpose, Magnesium chloride (MgCl₂.6H₂O), sodium dodecyl sulfate (SDS), sodium...
hydroxide (NaOH), Zinc acetate dihydrate (Zn (CH₃COO)₂·2H₂O), polyethylene glycol (PEG), urea (NH₂CONH₂), iron chloride tetrahydrate (FeCl₂·4H₂O) and ammonia were purchased from Sigma-Aldrich USA. A chemical method was applied to prepare nanoparticles (Parashar et al., 2020).

Well diffusion method
Standard culture (10⁵ CFU/ml) was aseptically disseminated on Mueller Hinton agar. The well-borer was drilling 6-8 mm holes. The suspension of antibiotic-coated nanoparticles was made individually for each nanoparticle that was poured into wells and incubated for 24 h at 37 °C the formation of inhibition zones around wells showed the preparation’s antibacterial activity (Anwar et al., 2020).

Minimum inhibitory concentration (MIC)
A standard inoculum (10⁵ CFU/mL) was used in the broth microdilution procedure to determine the MIC. Mueller Hinton broth in 96 wells microtitration plate was filled. Each preparation was 2-fold diluted, starting at 500 mg/mL, with one positive control and one negative control. For a total of five isolates, the test was performed in triplicate. At 37 °C, the plates were incubated for 24 h, and optical density (OD) readings were determined using a spectrophotometer at 590mm wavelength before and after incubation. The lowest concentration showing inhibition of growth was termed minimum inhibitory concentration (Anwar et al., 2020).

Statistical analysis
The prevalence was computed using the (Thrusfield, 2018) formula. When comparing the means of two groups, the student t-test was used, while ANOVA for more than three groups and tukey test to find significance among groups were applied on quantitative data. SPSS version 22 of the computer program was used at 5% probability.

RESULTS

Prevalence of S. aureus and MRSA
Prevalence of S. aureus from the study area was noted to be 24.56% while minimum was found as 17.14% to the highest as 25.71%. MRSA was found 16.07% in the study area with the lowest being 0% and the highest noted to be 25% (Table I). Study showed non-significant association (p>0.05) of farms with Staphylococcus aureus and same was observed in case of methicillin resistant Staphylococcus aureus.

Antibiotic susceptibility
Antibiogram of the current study found higher percentages of intermediate isolates. Unusual than routine susceptibility profile was also observed in case of antibiotics considered as solution therapy like linezolid and vancomycin (Table II). Highest resistant S. aureus were noted against gentamicin (50%) and chloramphenicol (50%) followed by enrofloxacin (40%), and septran (33%), vancomycin (30%), levofloxacin (30%), cefoxitin (30%). The study noted higher prevalence of intermediate susceptible S. aureus against septran (36.67%), vancomycin (30%), enrofloxacin (20%), and fusidic acid (16.67%). Highest percentage of sensitive isolates were shown against linezolid (80%), and fusidic acid (70%).

Resistance modulation by nanoparticles
Zone of inhibition (ZOI)
Current study showed significantly highest zone of inhibition by ZnO followed by MgO and Fe₂O₃ showing 24.53±0.80, 16.48±0.95 and 13.15±1.39mm, respectively. Difference of zone of inhibition was noted to be significant (p<0.05) when ZnO compared with MgO and Fe₂O₃. Similarly, MgO was statistically significant different than those of ZnO and Fe₂O₃. Same goes for Fe₂O₃ in comparison with MgO and ZnO (Fig. 2).

Table I. Percentage prevalence of Staphylococcus aureus and methicillin resistant Staphylococcus aureus (MRSA) isolated from commercialairy cattle milk.

| Dairy farm | Sample tested (N) | Positive (n) | Cattle milk S. aureus | Cattle milk MRSA |
|------------|-------------------|-------------|----------------------|------------------|
| A          | 35                | 9           | 25.71                |                  |
| B          | 35                | 6           | 17.14                |                  |
| C          | 42                | 7           | 16.67                |                  |
| D          | 34                | 15          | 44.12                |                  |
| E          | 23                | 5           | 21.74                |                  |
| F          | 24                | 6           | 25.00                |                  |
| G          | 35                | 8           | 22.86                |                  |

| Positive (x) | Percentage % (s/n*100) | CI (95%) p value | Positive (x) | Percentage % (s/n*100) | CI (95%) p value |
|--------------|-------------------------|-----------------|--------------|-------------------------|-----------------|
| 2            | 22.2                    | 1.58-18.6       | 2            | 16.66                  | 0.51-14.54      |
| 1            | 14.28                   | 0.51-14.54     | 1            | 13.33                  | 1.58-18.6       |
| 0            | 0.0                     | 0.00            | 1            | 16.66                  | 0.51-14.54     |
| 2            | 25                      | 1.58-18.6      |              |                         |                 |

Resistance Modulation of Staphylococcus aureus Isolates of Dairy Cattle
Table II. Antibiogram of *Staphylococcus aureus* isolated from dairy cattle milk.

| Antibiotics          | R    | I    | S    |
|----------------------|------|------|------|
| Vancomycin (VA30)    | 30.00| 30.00| 40.00|
| Levofloxacin (LEV)   | 30.00| 10.00| 60.00|
| Cefoxitin (CXT30)    | 30.00| 10.00| 60.00|
| Linezolid (LNZ30)    | 10.00| 10.00| 80.00|
| Enrofloxacin (ENR10) | 40.00| 20.00| 40.00|
| Gentamicin (CN10)    | 50.00| 10.00| 40.00|
| Fusidic acid (FA10)  | 13.33| 16.67| 70.00|
| Septran (SXT25)      | 33.33| 36.67| 30.00|
| Chloramphenicol (C30)| 50.00| 16.67| 33.33|

R, resistant; I, Intermediate; S, sensitive.

Comparison of incubation intervals for significant change in MIC was found different in all three nanoparticles (Fig. 3). ZnO showed significant difference of MIC when 4<sup>th</sup> incubation was compared with 8<sup>th</sup>, and 12<sup>th</sup> h of incubation. Similarly, 8<sup>th</sup> h of incubation was non-significant difference with 16<sup>th</sup> h of incubation. 12<sup>th</sup> h of incubation proved to be non-significantly different with all incubation periods. In case of MgO, significant difference started at/after 8<sup>th</sup> h of incubation while starting from 12<sup>th</sup> h onwards significant difference was not found particularly 16<sup>th</sup>, 20<sup>th</sup> and 24<sup>th</sup> h of incubation was non-significant with each other but significantly lower than those of 4<sup>th</sup> and 8<sup>th</sup> h incubation. Considerable variation in MICs was noted among different time intervals in case of Fe<sub>2</sub>O<sub>3</sub> in that significant difference started right after 4<sup>th</sup> h incubation. Middle order incubation h (12, 16, 20) were non-significant to each other and similarly 16<sup>th</sup> h onwards was non-significant with each other but significantly differing with initial 3 incubation periods. These findings reflect potential antibacterial activity at various hours.
SEM image of ZnO nanoparticles is shown in Figure 1. (i) Polygonal star-like microparticles are observed in this figure. This star-like particles are aligned randomly and are not fused properly. Particle borders are distinct, and each particle is made up of several spikes that are linked at the center, much like a star. The length of spikes is not equal. Every star-like particle is about 4-8 μm in diameter. Spikes’ terminal ends are blunt rather than pointy. Rod-shaped nanoparticles of Fe_2O_3 are observed in Figure 1. (ii) These rod-shaped particles are randomly aligned with each other. The length of the rods is around 2 μm while the width is around 100 nm. The terminal ends of rods are not pointed. It seems that rods have emerged from spherical nanoparticles because few spherical-shaped minute particles have adhered to the surface of rods. The rods are not aggregated so their surface is available for interacting with the environment. SEM images of MgO nanoparticles synthesized by the hydrothermal method are given in Figure 1. (iii) The product is comprised of spherical/oval-shaped nanoparticles which are fully dispersed. Aggregation is not observed in this image. The size of MgO nanoparticles lies in the 30-80 nm range. Contrast is not observed which shows that particles are compact and not hollow from the inside.

**DISCUSSION**

Contrary to the findings of current study no MRSA was detected by Ren et al. (2020) while S. aureus (41.5%) was found higher than our findings. On the other hands, current study is closely related to the findings of Li et al. (2017) who reported 23.6% prevalence of S. aureus while contrary to current study were findings of (Wang et al., 2018) who reported 6.3% S. aureus (Liu et al., 2017) reported 61.1% of S. aureus. Similarly, Turkey reported 83% S. aureus in raw milk (Bartolomeoli et al., 2009) while in Malaysia 66.7% (André et al., 2008), 56% in Brazil (Gundogan and Avci, 2014), Iran 12.4% (Jamali et al., 2015) and Italy reported 41.0% (Traversa et al., 2015) of S. aureus. In many parts of the world, milk and dairy products are contaminated by S. aureus, especially those strains carrying an MDR phenotype and possessing the ability to produce biofilms and toxins (Cavicchioli et al., 2015; Wang et al., 2018). Dairy products contaminated with S. aureus have led to food-borne poisoning outbreaks, demonstrating the public health significance of S. aureus (Rong et al., 2017). In addition, dairy farms suffer economic losses as a result of mastitis, since the disease lowers milk production, raises health care costs, and increases culling rates (Hennekinne et al., 2012). Susceptibility to different antibiotics was found 11.1% and 7.41%, against gentamicin and chloramphenicol, respectively (Liu et al., 2017). Higher resistance against vancomycin in current study was in line with findings of (Liu et al., 2021). Jamali et al. (2015) showed 5.5% of isolates resistant to cefotaxin. The lack of success of S. aureus therapy can be explained by its capacity for intracellular persistence within phagocytes as well as its antimicrobial resistance.

Antibacterial activity in terms of zone of ZOI of ZnO of current study was in line with findings of previous studies (Banoe et al., 2010; Jagathesan and Rajiv, 2018). The latter noted 23 mm ZOI in case of ZnO against S. aureus which is bit higher than our study. Similarly, studies regarding MIC values of nanoparticles against S. aureus showed similarities while somewhere contradiction was also found. The variation might be due to the differences in the study of S. aureus as well as test conditions (Sadiq et al., 2017). In another study, pathogens were completely inactivated after 12 h of incubation (Akbar et al., 2019) which is coinciding findings of current study. According to Jones et al. (2008), ZnO-NPs showed a significant decrease in bacterial viability compared to other metallic nanoparticles. According to Ren et al. (2009), ZnO- and CuO-NPs show distinct antimicrobial activity in a range of concentrations. Nanoparticles made from zinc oxide, or ZnO, are biocompatible and safe molecules, and they are primarily used as a means to deliver drugs (Vickers, 2017). It is speculated that nanoparticles hold unique mechanism of action different to those of antibiotics and exhibit promising results against (Fisher et al., 2018).

**CONCLUSION**

S. aureus isolated from commercial dairy was found...
to be multi-drug resistant. Gentamicin, enrofloxacin, levofloxacin, and vancomycin were found least effective compared to other antibiotics. Metallic nanoparticles zinc oxide, magnesium oxide, and ferric oxide have shown antibacterial activity against multiple drug resistance*S. aureus* both in well diffusion and broth microdilution assay. Metallic nanoparticles showed antibacterial activity by staging MgO to be more effective followed by Fe$_2$O$_3$.

S. aureus antibacterial activity against multiple drug resistance oxides, magnesium oxide, and ferric oxide have shown levofloxacin, and vancomycin were found least effective to be multi-drug resistant. Gentamicin, enrofloxacin, levofloxacin, and vancomycin were found least effective compared to other antibiotics. Metallic nanoparticles zinc oxide, magnesium oxide, and ferric oxide have shown antibacterial activity against multiple drug resistance *S. aureus* both in well diffusion and broth microdilution assay. Metallic nanoparticles showed antibacterial activity by staging MgO to be more effective followed by Fe$_2$O$_3$ and ZnO. The study proposes an *in-vivo* trial for dose optimization and validation of safety parameters.

**Statement of conflict of interest**

The authors have declared no conflict of interest.

**REFERENCES**

Akbar, A., Sadiq, M.B., Ali, I., Muhammad, N., Rehman, Z., Khan, M.N., and Anal, A.K., 2019. Synthesis and antimicrobial activity of zinc oxide nanoparticles against foodborne pathogens *Salmonella typhimurium* and *Staphylococcus aureus*. Biocatal. Agric. Biotechnol., 17: 36-42. https://doi.org/10.1016/j.bcab.2018.11.005

André, M.C.D., Campos, M.R.H., Borges, L.J., Kipnis, A., Pimenta, F.C., and Serafim, A.B., 2008. Comparison of *Staphylococcus aureus* isolates from food handlers, raw bovine milk and Minas Frescal cheese by antibiogram and pulsed-field gel electrophoresis following SmaI digestion. *Fd. Contr.*, 19: 200-207. https://doi.org/10.1016/j.foodcont.2007.03.010

Anwar, M.A., Aqib, A.I., Ashfaq, K., Deeba, F., Khan, M.K., Khan, S.R., Mazumdar, I., Shoaib, M., Naseer, M.A., Riaz, T., Tanveer, Q., Sadiq, M., Lodhi, F.L. and Ashraf, F., 2020. Antimicrobial resistance modulation of MDR *E. coli* by antibiotic coated ZnO nanoparticles. Microb. Pathog., 148: 104450.

Banoee, M., Seif, S., Nazari, Z.E., Jafari-Fesharaki, P., Shahverdi, H.R., Moballegh, A., and Shahverdi, M.K., 2010. ZnO nanoparticles enhanced antibacterial activity of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. *J. Biomed. Mater. Res. B Appl. Biomater.*, 93: 557-561. https://doi.org/10.1002/jbm.b.31615

Bartolomeoli, I., Maifreni, M., Frigo, F., Uri, G., and Marino, M., 2009. Occurrence and characterization of *Staphylococcus aureus* isolated from raw milk for cheese making. *Int. J. Dairy Technol.*, 62: 366-371. https://doi.org/10.1111/j.1471-0307.2009.00498.x

Bergey, D.H. and Holt, J.G., 1994. *Bergey’s manual of determinative bacteriology*. 9th Edition, Williams & Wilkins, Baltimore, Maryland.

Cavicchioli, V., Scatamburlo, T., Yamazi, A., Pieri, F., and Nero, L., 2015. Occurrence of *Salmonella, Listeria monocytogenes*, and enterotoxigenic *Staphylococcus* in goat milk from small and medium-sized farms located in Minas Gerais State, Brazil. *J. Dairy Sci.*, 98: 8386-8390. https://doi.org/10.3168/jds.2015-9733

CLSI, 2018. Performance standards for antimicrobial susceptibility testing performance standards for antimicrobial susceptibility testing suggested citation. CLSI Document M02-A11.

Dizaj, S.M., Lotfipour, F., Barzegar-Jalali, M., Zarrintan, M.H., and Adibkia, K., 2014. Antimicrobial activity of the metals and metal oxide nanoparticles. *Mater. Sci. Eng. C*, 44: 278-284.

Fisher, E.L., Otto, M., and Cheung, G.Y., 2018. Basis of virulence in enterotoxin-mediated staphylococcal food poisoning. *Front. Microbiol.*, 9: 436. https://doi.org/10.3389/fmicb.2018.00436

Foster, T.J., Geoghegan, J.A., Ganesh, V.K., and Höök, M., 2014. Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. *Nat. Rev. Microbiol.*, 12: 49-62. https://doi.org/10.1038/nrmicro3161

Geiser, M., 2010. Update on macrophage clearance of inhaled micro-and nanoparticles. *J. Aerosol Med. Palm. Drug Delivery*, 23: 207-217. https://doi.org/10.1089/jamp.2009.0797

Gomes, F., and Henriques, M., 2016. Control of bovine mastitis: Old and recent therapeutic approaches. *Curr. Microbiol.*, 72: 377-382.

Gundogan, N., and Avci, E., 2014. Occurrence and antibiotic resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey. *Int. J. Dairy technol.*, 67: 562-569. https://doi.org/10.1111/1471-0307.12149

Hennekinne, J.A., De Buys, M.L., and Dragacci, S., 2012. *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. *FEMS Microbiol. Rev.*, 36: 815-836. https://doi.org/10.1111/j.1574-6976.2011.00311.x

Jagathesan, G., and Rajiv, P., 2018. Biosynthesis and characterization of iron oxide nanoparticles using *Eichhornia crassipes* leaf extract and assessing their antibacterial activity. *Biocatal. Agric. Biotechnol.*, 13: 90-94. https://doi.org/10.1016/j.bcab.2017.11.014

Jamali, H., Paydar, M., Radmehr, B., Ismail, S., and Dadransia, A., 2015. Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from
raw milk and dairy products. *Fd. Contr.*, **54**: 383-388. https://doi.org/10.1016/j.foodcont.2015.02.013

Javed, M.U., Ijaz, M., Fatima, Z., Anjum, A.A., Aqib, A.I., Ali, M.M., and Ghaffar, A., 2021. Frequency and antimicrobial susceptibility of methicillin and vancomycin-resistant *Staphylococcus aureus* from bovine milk. *Pak. Vet. J.*, **41**: 463-468.

Jin, T., and He, Y., 2011. Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. *J. Nanopart. Res.*, **13**: 6877-6885.

Jones, N., Ray, B., Ranjit, K.T., and Manna, A.C., 2008. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS Microbiol. Lett.*, **279**: 71-76.

Kalhapure, R.S., Sonawane, S.J., Sikwal, D.R., Jadhav, M., Rambarose, S., Mocktar, C., and Govender, T., 2015. Solid lipid nanoparticles of clotrimazole silver complex: an efficient nano antibacterial against *Staphylococcus aureus* and MRSA. *Colloids Surf. B Biointerf.*, **136**: 651-658. https://doi.org/10.1016/j.colsurfb.2015.10.003

Li, T., Lu, H., Wang, X., Gao, Q., Dai, Y., Shang, J., and Li, M., 2017. Molecular characteristics of *Staphylococcus aureus* causing bovine mastitis between 2014 and 2015. *Front. Cell. Infect. Microbiol.*, **7**: 127. https://doi.org/10.3389/fcimb.2017.00127

Liu, H., Li, S., Meng, L., Dong, L., Zhao, S., Lan, X., and Zheng, N., 2017. Prevalence, antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from dairy herds in northern China. *J. Dairy Sci.*, **100**: 8796-8803. https://doi.org/10.3168/jds.2017-13370

Liu, Y., Yang, K., Jia, Y., Shi, J., Tong, Z., Fang, D., and Xiao, X., 2021. Gut microbiome alterations in high-fat-diet-fed mice are associated with antibiotic tolerance. *Nat. Microbiol.*, **6**: 874-884.

Muhammad, G., Naureen, A., Asi, M.N., and Saqib, M., 2010. Evaluation of a 3% surf solution (surf field experiment) for the diagnosis of subclinical bovine and bubaline mastitis. *Trop. Anim. Hlth. Prod.*, **42**: 457-464.

Parashar, M., Shukla, V.K., and Singh, R., 2020. Metal oxides nanoparticles via sol–gel method: a review on synthesis, characterization and applications. *J. Mater. Sci. Mater. Electron.*, **31**: 3729-3749.

Ren, G., Hu, D., Cheng, E.W., Vargas-Reus, M.A., Reip, P., and Allaker, R.P., 2009. Characterisation of copper oxide nanoparticles for antimicrobial applications. *Int. J. Antimicrob. Agents*, **33**: 587-590. https://doi.org/10.1016/j.ijantimicag.2008.12.004

Ren, Q., Liao, G., Wu, Z., Lv, J., and Chen, W., 2020. Prevalence and characterization of *Staphylococcus aureus* isolates from subclinical bovine mastitis in southern Xinjiang, China. *J. Dairy Sci.*, **103**: 3368-3380. https://doi.org/10.3168/jds.2019-17420

Rong, D., Wu, Q., Xu, M., Zhang, J., and Yu, S., 2017. Prevalence, virulence genes, antimicrobial susceptibility, and genetic diversity of *Staphylococcus aureus* from retail aquatic products in China. *Front. Microbiol.*, **8**: 714. https://doi.org/10.3389/fmicb.2017.00714

Sadiq, M.B., Tarning, J., Aye Cho, T.Z., and Anal, A.K., 2017. Antibacterial activities and possible modes of action of *Acacia nilotica* (L.) Del. against multidrug-resistant *Escherichia coli* and *Salmonella*. *Molecules*, **22**: 47. https://doi.org/10.3390/atoms22010047

Sarwar, I., Ashar, A., Mahfooz, A., Aqib, A.I., Saleem, M.I., Butt, A.A., and Ilyas, A., 2021. Evaluation of antibacterial potential of raw turmeric, nano-turmeric, and NSAIDs against multiple drug resistant *Staphylococcus aureus* and *E. coli* isolated from animal wounds. *Pak. Vet. J.*, **41**: 209-214.

Shoaib, M., Rahman, S.U., Aqib, A.I., Ashfaq, K., Naveed, A., Kulyar, M.F.A., and Naseer, M.A., 2020. Diversified epidemiological pattern and antibiogram of mecA gene in *Staphylococcus aureus* isolates of pets, pet owners and environment. *Pak. Vet. J.*, **40**: 318-327.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., and Carmeli, Y., 2018. Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.*, **18**: 318-327.

Thrusfield, M., 2018. Veterinary epidemiology. John Wiley and Sons.

Tong, S.Y., Davis, J.S., Eichenberger, E., Holland, T.L., and Fowler, V.G., 2015. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clin. Microbiol. Rev.*, **28**: 603-661. https://doi.org/10.1128/CMR.00134-14

Traversa, A., Gariano, G., Gallina, S., Bianchi, D., Orusa, R., Domenis, L., and Decastelli, L., 2015. Methicillin resistance in *Staphylococcus aureus* strains isolated from food and wild animal carcasses in Italy. *Fd. Microbiol.*, **52**: 154-158. https://doi.org/10.1016/j.fdm.2015.07.012

Vickers, N.J., 2017. Animal communication: When I'm calling you, will you answer too? *Curr.
Biol., 27: R713-R715. https://doi.org/10.1016/j.cub.2017.05.064

Wang, W., Lin, X., Jiang, T., Peng, Z., Xu, J., Yi, L., and Baloch, Z., 2018. Prevalence and characterization of Staphylococcus aureus cultured from raw milk taken from dairy cows with mastitis in Beijing, China. Front. Microbiol., 9: 1123. https://doi.org/10.3389/fmicb.2018.01123