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Prevalence of livestock associated methicillin resistant *Staphylococcus aureus* (La-mrsa) in domestic livestock of Dharan, Nepal

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Abstract

Introduction: Methicillin Resistant *Staphylococcus aureus* (MRSA) infection has drawn a lot of attention since studies suggested that animals may act as reservoirs for human infection. Over the past 20 years, MRSA infections have become more prevalent worldwide. MRSA was initially only discovered in humans, but later it was discovered in animals as well.

Objectives: The study aimed to determine the prevalence of LA-MRSA in domestic livestock of Dharan, Nepal.

Methods: Overall, 320 skin swab samples of cattle (cows) were collected by swabbing the skin of cattle aseptically with a sterile cotton swab and the samples were transported to the laboratory in a cold chain. The samples were streaked in Mannitol salt agar(MSA) containing oxacillin concentration of 6mg/L and incubated at 37°C for 24 hours. *Staphylococcus aureus* colonies were identified based on cultural characteristics on MSA plates (golden yellow colonies), Gram's reaction, and positive results for coagulase and catalase test. The purified isolates of MRSA were subjected to antibiotic susceptibility tests and Biofilm formation.

Results: MRSA was found to be prevalent in 10% of cattle. Antibiotic-resistant tests reported that MRSA was found to be sensitive to chloramphenicol 12(37.5%) followed by ceftriaxone 12 (37.25%), gentamycin 9(28.12%), cefotaxime 4(12.5%), while 100% of MRSA were resistant to penicillin and vancomycin. The moderate biofilm-forming MRSA was 5(15.625%), followed by 3(9.375%) weak biofilm producers and 24(75%) were negative towards biofilm formation.

Conclusion: The prevalence of MRSA (10%) and Vancomycin-Resistant *Staphylococcus aureus* (100% VRSA) in cattle shows the need for regular surveillance. Chloramphenicol can be a treatment of choice for MRSA infections. However, emerging VRSA is a serious epidemiological issue that needs to be addressed properly. Therefore, Healthcare organizations must adopt precise criteria to control and prevent MRSA infection..

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1. Introduction

Staphylococci are gram-positive bacteria that are non-motile and do not produce spores. The capacity to manufacture coagulase, which clots the blood, distinguishes pathogenic staphylococci (Harris et. al., 2002). *Staphylococcus aureus (S. aureus)* is the most common pathogen which causes intra-mammary infections in animals, especially cattle and is one of the most important pathogens that is responsible for bovine mastitis (Bradley, 2002). MRSA strains (Methicillin-resistant *Staphylococcus aureus*) are *S. aureus* bacteria that have developed resistance to all Beta-lactam drugs.

MRSA strains have been found to be transmitted from animals to people, according to scientists (Markey et. al., 2008). Because many of the MRSA clonal lineages found in livestock were uncommon in MRSA isolates previously found in human hosts, the term "livestock-

associated MRSA" (LA-MRSA) was coined to distinguish these MRSA from the more common human hospital-acquired (HA-MRSA) and community-associated MRSA (CA-MRSA) (Koch et. al., 2013). LAMRSA CC938 was considered the only strain responsible for animal infection. In the last few decades, staphylococcal food poisoning has been reported as the third cause of foodborne illness in the *world* (Ateba et. al., 2010).

MRSA causes incurable intra-mammary infections and skin problems in cattle. It can induce deep-seated infections like endocarditis and osteomyelitis in extreme situations. MRSA gained zoonotic significance when scientists proposed that cattle might serve as reservoirs for human MRSA infection (Joshi and Devkota, 2014).

A total of 94 articles were eligible for inclusion in this meta-analysis. The pooled prevalence of MRSA was estimated to be 3.81% [95% confidence interval (95% CI) = 2.61–5.20] with significantly high *heterogeneity* (I^2 = 96.6%, p = 0.00). For the subgroup analysis among continents, the prevalence was highest in Asia (4.89%; 95% CI = 2.88–7.35) and lowest in South America (1.33%, 95% CI = 0.00–5.49). As for the year of publication, MRSA prevalence was highest in reports published from 2015 to 2018 (4.36%, 95% CI = 2.41–6.80) and lowest in reports published before 2015 (2.65%, 95% CI = 0.75–5.52). As for sample type, the prevalence of MRSA in cattle milk (3.91%, 95% CI = 2.64–5.39) was higher than that in other sample types (1.19%, 95% CI = 0.05–3.24). These three factors were not significantly associated with the pooled prevalence of MRSA (p > 0.05) (khanal et. al.,2022)

It has long been recognized that biofilms increase resistance to antimicrobial action from both external agents, such as antibiotics, and internal agents of the innate immune system, such as antimicrobial peptides (AMPs). (Craft et. al., 2019). Biofilms are aggregates of microbial cells surrounded by a matrix of exopolymers (Costerton et. al., 1999).

It is important to avoid unnecessary contact with the cattle to undertake proper hygienic precautions and also minimize the indiscriminate use of antibiotics to prevent the development of staphylococcal bacterial resistance. This study is important for veterinary and medical personnel to be aware of MRSA infection occurrence in animals. Similarly, it also provides the information to avoid the use of antibiotics without a doctor's prescription as well as encourages owners for regular checkups and proper vaccination for cattle. Therefore, the goal of the study was to determine the prevalence of livestock-associated MRSA, in the livestock of Dharan.

2. Materials and Method

2.1 Study site and study sample

This was a laboratory-based cross-sectional study carried out in Dharan Sub-metropolitan city from March to December 2020. Dharan is a small city located in Sunsari district (Latitude: 26° 48' 44.93" N; Longitude: 87° 17' 0.78" E), Sunsari Nepal with the 2112-hectare area and located in the eastern Terai of Nepal stretching from the edge of northern Mahabharat hill range up to the Charkoshe Jhadi in south separating from the southern Terai. (Figure 1). A simple random sampling technique was followed to select cattle. The cattle were selected by lottery method after the sites were randomly selected**.** A total of 320 skin swab samples were collected from different household-rearing cattle of the Dharan sub-metropolitan city.

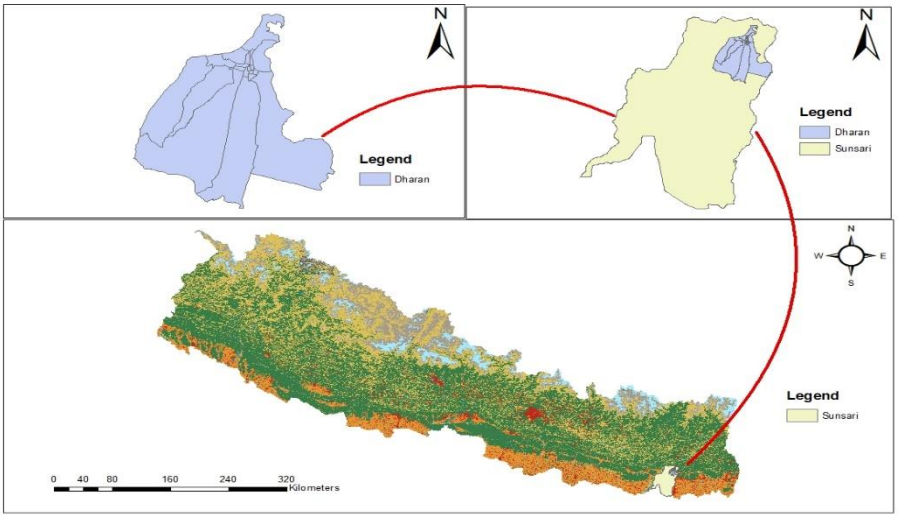

Figure 1. Map of study area of Dharan, Sunsari. (Source: Arc map GIS 10.7)

2.2 Sample collection and transportation

The skin swab sample was collected under aseptic conditions with a sterile cotton swab. Skin swabs were collected by rolling the sterile swab on the back skin 2-3 times and dispensed in a sterile 1 mL nutrient broth (HiMedia, India) tube. The samples were preserved for 1 hour in a sterile vial containing nutrient broth under cold conditions and brought to the microbiology laboratory of the Central Campus of Technology, Dharan.

2.3. *Isolation and identification of Staphylococcus aureus*

The isolation of LA-MRSA from skin swabs was conducted as described by Smith et al, (2009). The cotton swab-containing sample was swabbed in a Mannitol Salt Agar (MSA) (HiMedia, India) plate containing 6 mg/l oxacillin concentration and incubated for 24 hours at 37^{o}C.

2.4 Identification of MRSA

Evidence from Gram's staining, catalase test, coagulase test, and colony features allowed for the identification of MRSA. Growth of *S. aureus* on an MSA plate containing oxacillin of 6 mg/l gave the preliminary confirmation about MRSA. A coagulase test and catalase test were performed for further confirmation. MRSA was confirmed by using the cefoxitin disc diffusion method. For this 24 hours old *S. aureus* cultures were prepared in Nutrient broth (HiMedia, India) and turbidity was maintained with the 0.5 McFarland turbidity standard. This culture was swabbed onto the Muller-Hilton agar (MHA) (HiMedia, India) plate and allowed to stand for 5 minutes, and then the cefoxitin disc (30µg) was kept on it. The plates were incubated aerobically at 37^{o}C for 24 hours. The plates were observed, and the zone of inhibition was measured. The isolates which has a zone of inhibition ≤21mm was considered a methicillin-resistant *S. aureus* as recommended by CLSI guidelines (CLSI 2012).

2.5 *Antibiotic susceptibility test*

The positive samples of MRSA were subjected to an antibiotic susceptibility test by the Kirby-Bauer disc diffusion technique using Muller-Hilton agar media (HiMedia) as recommended by CLSI guidelines (CLSI 2012). The positive samples for MRSA were inoculated in nutrient broth at 37^{o}C for 24 hours to achieve the recommended 0.5 McFarland turbidity. The MHA was prepared and plated out. Then it was swabbed with fresh culture with a sterile cotton swab. The antibiotic discs that are cefotaxime (Ctx,30mcg), chloramphenicol (C, 30mcg), ceftriaxone (Ctr, 30mcg), erythromycin (E, 15mcg), clindamycin (Cd, 2mcg), gentamycin (Gen, 10mcg), penicillin (P, 10mcg), and

vancomycin (Va, 30mcg) were placed on a plate with the help of sterile forceps and allowed to stand for 15 minutes for diffusion, and then incubated at 37°C for 24 hours. After 24 hours, the plates were observed, and the zone of inhibition was measured. Then the results were interpreted as susceptible, intermediate, and resistant according to the CLSI Diffusion Supplement Table (CLSI 2012).

2.6 *Detection* of Biofilm

The quantification of biofilm by the Tissue culture plate method was performed according to Christensen et al (1985). In this method, 5ml of overnight culture of MRSA was prepared. Then, 100µl of diluted culture was inoculated in a well of tissue culture plate containing Tryptic Soy broth (TSB) (HiMedia, India) with 5% glucose. The plate was incubated at 37ºC for 24 hours. The unbound cell was discarded and washed several times by Phosphate Buffer Saline (HiMedia, India) (pH-7.2). Exactly 125µl of 0.1% crystal-violet solution (HiMedia, India) was added to wells and left for 10-15 minutes of incubation. The plate was washed and left inverted for drying. The quantitative determination was performed by solubilizing the biofilm by adding 125µl of 30% acetic acid to each well and incubating the plate for 10-15 minutes at room temperature and transferring it to another Tissue culture plate and reading the absorbance at 450 nm by ELISA plate reader (Loncare LR-620 microplate reader, Medical Technology Co., Ltd.). Interpretation is made on optical density (OD) by subtracting OD of control wells from OD of test wells. The optical density (ODs) of each strain was obtained by the arithmetic mean of the absorbance of three wells and this value was compared with the mean absorbance of negative controls (ODnc). The following classification was used for the determination of biofilm formation: no biofilm production (ODs≤ODnc), weak biofilm production (ODnc<ODs≤2.ODnc), moderate biofilm production (2.ODnc<ODs≤4.ODnc), and strong biofilm production (4.ODnc<ODs) (Stepvanovic et. al., 2007).

2.7 *Quality Control and data analysis*

During medium preparation, sample collection, sample processing, and culture identification, the entire aseptic environment was maintained. As a positive control, the *S. aureus* ATCC 25923 strain was used. The data was documented and tabulated in MS Excel 2010.

3. Results and Discussion

3.1 *Study population*

Out of 320 skin swab samples, 32(10%) samples were MRSA-positive.

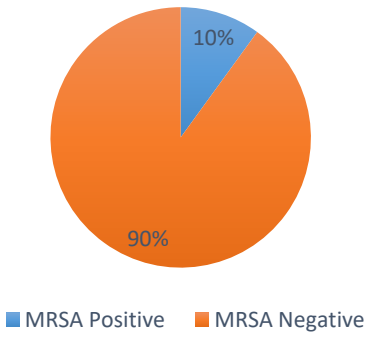

Figure 2: Prevalence of MRSA from the sample.

3.2 *Prevalence of MRSA in study Sites*

In this study, 122 samples were collected from Budha Subba site, where 8 (6.56%) were MRSA-positive. Similarly, 78 samples were from Purano Hattisar site in which 6 (7.69%) were MRSA positive, 62 samples were from Pindeswori site in which 10 (16%) were MRSA positive. 11 samples were from Pritivi Chowk site in which 2(18%) were MRSA positive and 42 samples were from Panbari site in which 6 (14%) were positive by the incidence of MRSA (Table 1).

Table 1: Prevalence of MRSA from Different sites

S.N	Location	No. of samples from cattle	No. of the positive sample MRSA
1	Buddha subba	122	8 (6.56%)
2	Purano hattisar	78	6 (7.69%)
3	Pindeswori	62	10(16%)
4	Pritivi chowk	11	2(18%)
5	Panbari	42	6(14%)
	Total	320	32

3.3 Antibiotic susceptibility pattern of MRSA isolates

In this study, most of the isolates were found to be sensitive to chloramphenicol 12(37.5%) followed by ceftriaxone 12 (37.25%), gentamycin 9(28.12%), cefotaxime 4(12.5%) while 100% MRSA was resistant to penicillin and vancomycin (Table 2).

Table 2: Antibiotic susceptibility pattern of MRSA isolates

SN	Antibiotics	Disc Content (mcg)	Resistant	Intermediate	Sensitive
1	Erythromycin	15	29 (90.62%)	2 (6.25%)	1 (3.7%)
2	Gentamycin	10	23 (71.87%)	—	9 (28.12%)
3	Tetracycline	30	26(81.25%)	5 (15.62%)	1 (3.7%)
4	Vancomycin	30	32 (100%)	—	
5	Cefotaxime	30	18(56.25%)	10 (31.25%)	4 (12.5%)
6	Penicillin	10	32(100%)	—	
7	Chloramphenicol	30	16 (50%)	4 (12.5%)	12 (37.5%)
8	Ceftriaxone	30	9 (28.12%)	12(37.25%)	12(37.25%)
9	Clindamycin	2	30(93.75%)	—	2 (6.25%)

3.4 Screening Biofilm forming MRSA

In this study, 5(15.62%) of 32 MRSA isolates were moderate biofilm producer, followed by 3(9.37%) weak biofilm former and 24(75%) were non-biofilm forming isolates (Table 3).

Table 3: Screening biofilm-producing MRSA.

Biofilm formation	Total (percentage)
High	--
Medium	5(15.625%)
Weak	3(9.375%)
None	24(75%)

Staphylococcus aureus is a pathogen that is commonly isolated in most microbiological laboratories (Ansari et. al., 2014). It causes a variety of infections, including superficial skin infections, food poisoning, osteomyelitis, and septicemia (Forbes et. al., 2007). The study is aimed at determining the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) from the skin of cattle and to determine the Biofilm production of the MRSA isolates.

According to a study by Hansen et al (2019), out of 620 calves' skin samples, 11.8% were found to be MRSA which determined a low prevalence of MRSA. Similar findings were obtained in the present study. The result obtained agrees with the findings of Vanderhaeghen et al (2010) who reported an MRSA prevalence of 9.3%. Similarly, another study done by Schnitt et al (2020) found a 22.7% prevalence of MRSA. The prevalence of MRSA was 3.4% in one study by Seung Hyun Back et al (2019). Similarly, a higher prevalence of MRSA was found in the study done by Kumar et al (2017) which showed a 31.43% incidence of MRSA. Similarly, a low prevalence (0.7%) of MRSA harboring in the nose and skin of cattle has been reported in Thailand (Patchnee et. al., 2014). However, the difference in prevalence may be due to the fact that our samples were collected from a small geographical area within a shorter period compared to those reported elsewhere. MRSA has gained a lot of interest as a zoonotic organism in recent years when research revealed that animals might serve as reservoirs for human MRSA infection (Joshi et. al., 2014).

In the present study, the antibiotic susceptibility test reported that MRSA isolates were sensitive to chloramphenicol 37.5% followed by ceftriaxone 37.25%, gentamycin 28.12%, cefotaxime 12.5% while MRSA was resistant (93.75%) to Clindamycin and 100% were resistant to penicillin and vancomycin. Kumar et.al, (2017) showed an Antimicrobial susceptibility test of MRSA resistant to penicillin (88%) and, cefoxitin (75%), cotrimoxazole (62%), which is lower than the present study. Patchnee et al, (2014) showed Antimicrobial sensitivity tests of MRSA isolates were 100% resistant to clindamycin which is higher than the present study, however, 100% of isolates were susceptible to vancomycin which is dissimilar to the present study. Emerging VRSA isolates are a serious concern as Vancomycin is the only drug of choice to treat MRSA infection.

Antibiotic-resistant *Staphylococcus aureus* isolates impose a challenge to both veterinary, health professions and dairy cattle producers because they have a negative impact on therapy (Brouillette et. al., 2017). Girmay et al, (2020) found, out of 220 dairy cows 29.08% were positive for bovine mastitis. Out of which 32.81% were coagulase-positive *S. aureus*. In the 1970s, MRSA was discovered in a veterinary environment after drug-resistant isolates from cattle were discovered in Belgium (Devriese et. al., 1972).

Biofilm can lead to persistent infection because the

cells within the biofilm are very resistant to the host immune system and antimicrobial agents (Song et. al., 2017). In this study, moderate and weak biofilm-producing MRSA were identified. The biofilm-producing ability must have assisted in antibiotic drug resistance though many other factors are also responsible for antibiotic drug resistance. Biofilm-forming MRSA infection possesses great clinical challenges. Therefore, knowledge of the biofilm-producing ability of MRSA is necessary for prescribing drugs that are found sensitive to biofilm-forming isolates.

4. Conclusion

In this study, 10% of cattle were found to be carrier of LA-MRSA, and 100% isolated MRSA were VRSA. Isolates of MRSA were most sensitive to chloramphenicol which can be a drug of choice in MRSA infection. VRSA prevalence is a significant epidemiological problem that requires appropriate attention. MRSA carriage in animals and humans appears great threat to effective antimicrobial treatment. Therefore, clinicians and healthcare institutions must adopt precise guidelines to prevent and manage MRSA infection.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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References

Asli, A., Brouillette, E., Ster, C., Ghinet, M. G., Brzezinski, R., Lacasse, P., ... & Malouin, F. (2017). Antibiofilm and antibacterial effects of specific chitosan molecules on Staphylococcus aureus isolates associated with bovine mastitis. *PloS one*, *12*(5), e0176988.

Ansari, S., Nepal, H. P., Gautam, R., Rayamajhi, N., Shrestha, S., Upadhyay, G., & Chapagain, M. L. (2014). Threat of drug resistant Staphylococcus aureus to health in Nepal. *BMC infectious diseases*, *14*(1), 1-5.

Ateba, M., Mbewe M. S., Moneoang, and C.C., Bezuidenhout, Antibiotic – resistant *Staphylococcus aureus* isolated from milk in the Mafikeng area, north west province, south Africa, *South African Jouranl of Science*, vol 106 pp 12-13 2010.

Back, S. H., Eom, H. S., Lee, H. H., Lee, G. Y., Park, K. T., & Yang, S. J. (2020). Livestock-associated methicillin-resistant Staphylococcus aureus in Korea: antimicrobial resistance and molecular characteristics of LA-MRSA strains isolated from pigs, pig farmers, and farm environment. *Journal of veterinary science*, *21*(1).

Bradley, A. J. (2002). Bovine mastitis: an evolving disease. *The veterinary journal*, *164*(2), 116-128.

Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *science*, *284*(5418), 1318-1322.

Catry, B., Van Duijkeren, E., Pomba, M. C., Greko, C., Moreno, M. A., Pyörälä, S., & Torren-Edo, J. (2010). Reflection paper on MRSA in food-producing and companion animals: epidemiology and control options for human and animal health. *Epidemiology & Infection*, *138*(5), 626-644.

Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton, D. M., & Beachey, E. H. (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *Journal of clinical microbiology*, *22*(6), 996-1006.

Clinical and laboratory standard institute. (2012). Performance standards for antimicrobial disc

susceptibility tests, twentieth supplement;32(3): M100- S21.

Craft, K. M., Nguyen, J. M., Berg, L. J., & Townsend, S. D. (2019). Methicillin-resistant Staphylococcus aureus (MRSA): antibiotic-resistance and the biofilm phenotype. *MedChemComm*, *10*(8), 1231-1241.

Devriese, L. A., Van Damme, L. R., & Fameree, L. (1972). Methicillin (cloxacillin)-resistant Staphylococcus aureus strains isolated from bovine mastitis cases. *Zentralblatt für Veterinärmedizin Reihe B*, *19*(7), 598-605.

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). Bailey and Scott's Diagnostic Microbiology 12th edition. Mosby Elsevier Publication, USA pp 842-846. urinary tract infection patients. *Int J Appl Sci Biotechnol*, *1*(1), 1-4.

Girmay, W., Gugsa, G., Taddele, H., Tsegaye, Y., Awol, N., Ahmed, M., & Feleke, A. (2020). Isolation and identification of methicillin-resistant Staphylococcus aureus (MRSA) from milk in shire dairy farms, Tigray, Ethiopia. *Veterinary medicine international*, *2020*.

Khanal, S., Boonyayatra, S., & Awaiwanont, N. (2022). Prevalence of methicillin-resistant Staphylococcus aureus in dairy farms: A systematic review and meta-analysis. *Frontiers in Veterinary Science*, *9*, 947154.

Hansen, J. E., Ronco, T., Stegger, M., Sieber, R. N., Fertner, M. E., Martin, H. L., & Pedersen, K. (2019). LA-MRSA CC398 in dairy cattle and veal calf farms indicates spillover from pig production. *Frontiers in Microbiology*, *10*, 2733.

Harris, L. G., Foster, S. J., & Richards, R. G. (2002). An introduction to Staphylococcus aureus, and techniques for identifying and quantifying S. aureus adhesins in relation to adhesion to biomaterials: review. *Eur Cell Mater*, *4*(3), 100-20.

Joshi, L. R., Tiwari, A., Devkota, S. P., Khatiwada, S., Paudyal, S., & Pande, K. R. (2014). Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in dairy farms of Pokhara, Nepal. *International Journal of Veterinary Science*, *3*(2), 87-90.

Joshi, L. R., & Devkota, S. P. (2014). Methicillin-resistant Staphylococcus aureus (MRSA) in cattle: epidemiology and zoonotic implications. *International Journal of Applied Sciences and Biotechnology*, *2*(1), 29-33.

Köck, R., Schaumburg, F., Mellmann, A., Köksal, M., Jurke, A., Becker, K., & Friedrich, A. W. (2013). Livestock-associated methicillin-resistant Staphylococcus aureus (MRSA) as causes of human infection and colonization in Germany. *PloS one*, *8*(2), e55040.

Markey, B., Leonard, F., Archambault, M., Cullinane, A., & Maguire, D. (2013). *Clinical veterinary microbiology e-book*. Elsevier Health Sciences.

Schnitt, A., Lienen, T., Wichmann-Schauer, H., Cuny, C., & Tenhagen, B. A. (2020). The occurrence and distribution of livestock-associated methicillin-resistant Staphylococcus aureus ST398 on German dairy farms. *Journal of Dairy Science*, *103*(12), 11806-11819.

Smith, T. C., Male, M. J., Harper, A. L., Kroeger, J. S., Tinkler, G. P., Moritz, E. D., & Diekema, D. J. (2009). Methicillin-resistant Staphylococcus aureus (MRSA) strain ST398 is present in midwestern US swine and swine workers. *Plos one*, *4*(1), e4258.

Song, M., Li, Q., Zhang, Y., Song, J., Shi, X., & Shi, C. (2017). Biofilm formation and antibiotic resistance pattern of dominant Staphylococcus aureus clonal lineages in China. *Journal of Food Safety*, *37*(2), e12304.

Stepanović, S., Vuković, D., Hola, V., Bonaventura, G. D., Djukić, S., Ćirković, I., & Ruzicka, F. (2007). Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *Apmis*, *115*(8), 891-899.

Kumar, A., Kaushik, P., Kumar, P., & Kumar, M. (2017). Prevalence of methicillin-resistant Staphylococcus aureus skin and nasal carriage isolates from bovines and its antibiogram. *Veterinary World*, *10*(6), 593.

Patchanee, P., Tadee, P., Arjkumpa, O., Love, D., Chanachai, K., Alter, T., ... & Tharavichitkul, P. (2014). Occurrence and characterization of livestock-associated methicillin-resistant Staphylococcus aureus in pig industries of northern Thailand. *Journal of Veterinary Science*, *15*(4), 529-536.

Vanderhaeghen, W., Cerpentier, T., Adriaensen, C., Vicca, J., Hermans, K., & Butaye, P. (2010). Methicillin-resistant Staphylococcus aureus (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. *Veterinary microbiology*, *144*(1-2), 166-171.