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Multiple antibiotic resistance and virulence factors of *Staphylococcus aureus* strains isolated from dairy farms in South Sulawesi, Indonesia

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Abstract. *Juwita S, Indrawati A, Damajanti R, Safika, Mayasari NLPI. 2022. Multiple antibiotic resistance and virulence factors of* Staphylococcus aureus *strains isolated from dairy farms in South Sulawesi, Indonesia. Biodiversitas 23: 1015-1022.* Antimicrobial resistance (AMR) is an important issue affecting human and animal health worldwide. This study aimed to investigate antibiotic resistance and determine the virulence factors of *S. aureus* isolated from the dairy farms in South Sulawesi, Indonesia. Thirty-one isolates of *S. aureus* were tested for sensitivity to 9 types of antibiotics using the Kirby-Bauer disk diffusion method. The analysis of antibiotic resistance and virulence genes in *S. aureus* isolates was performed by the conventional PCR method. The results showed that *S. aureus* isolates from human samples were resistant to penicillin G (PEN) (86%), ampicillin (AMP) (86%), oxacillin (OXA) (14%), cefoxitin (FOX) (14%), tetracycline (TE) (43%) and ciprofloxacin (CIP) (14%). *Staphylococcus aureus* isolates from the animal samples were resistant to penicillin G (PEN) (50%), ampicillin (AMP) (50%), tetracycline (TE) (15%), and erythromycin (5%). Meanwhile, *S. aureus* isolates from dangke were resistant to penicillin G (PEN) and ampicillin (AMP) (50% each). Antimicrobial resistance genes for *blaTEM* (83%), *mecA* (17%), and *tetA* (100%) were detected in *S. aureus* isolates from human samples, whereas those for *blaTEM* (90%) and *tetA* (100%) were detected in isolates from animal samples. Meanwhile, the genes for *blaTEM* (100%) were detected in isolates from dangke. A total of 19 *S. aureus* isolates harbored the virulence gene for *fnbA* (26%), *clfA* (58%), *hla* (58%), and *tst* (21%). The use of antibiotics in humans and animals needs to be implemented properly in local communities to prevent the spread of antibiotic resistance. The presence of the *tst* gene in raw milk is essential for consumer protection against the risk of toxic shock syndrome.

Keywords: Antibiotic resistance genes, dairy farm, one health, *Staphylococcus aureus*, virulence factor gene

INTRODUCTION

Staphylococcus aureus is one of the pathogenic bacteria that can cause disease in humans (Liana et al. 2015). *Staphylococcus aureus* is considered an important cause of zoonotic diseases and poses a serious threat to human and animal health (Rahman et al. 2020); it is also known as a foodborne pathogen, causing food poisoning outbreaks worldwide (Fetsch and Johler 2018). *Staphylococcus aureus* is known to produce a wide array of virulence factors such as enterotoxins, hemolysins, and surface protein adhesins, which contribute to the type and severity of staphylococcal infections (Cheung et al. 2021). The risk of *S. aureus* infection spreading from animals to humans through handling, close contact, and consumption of *S. aureus*-infected animal products is a direct threat to public health (Klous et al. 2016; Dittmann et al. 2017).

Antimicrobial resistance (AMR) is a significant health issue that impacts human and animal health worldwide (Pokharel et al. 2020). The effects of AMR include severe illness, increased healthcare expenses, higher second-line medicine expenses, prolonged hospital admissions, and treatment failures (Dadgostar et al. 2019). The history of antibiotic use is the primary driver behind AMR, resulting in selection pressure that is specific to the antibiotic type and bacterial species (Munita and Arias 2016). AMR can develop naturally due to antimicrobial exposure during the treatment of veterinary and human clinical cases (Kumar et al. 2021). AMR transmission has also been linked to food production and animal husbandry practices (Sharma et al. 2018).

Treatment of *S. aureus* infections with antibiotics often results in developing resistance to any antibiotics (Foster 2017). The incidence of *S. aureus* resistance to antibiotics varies according to the region (Deyno et al. 2017; Mekonnen et al. 2018; Wang et al. 2018). The acquisition and spread of resistance generally occur through horizontal or lateral gene transfer (HGT) (Ma et al. 2021). HGT plays an essential role in the evolution of *S. aureus*. Indeed, a variety of antibiotic resistance genes and virulence factors are found in a series of mobile genetic elements (MGEs) such as integron (In), transposons (Tn), bacteriophages, insertion sequences (IS), pathogenicity islands (PI), and integrative conjugative elements (ICEs) (Naito and Pawlowska 2016; Partridge et al. 2018; Cheung et al. 2021).

Antibiotic resistance is driven by antibiotic use and abuse in animal, human, and environmental sectors, as well as the spread of resistant bacteria and determinants within and between these sectors worldwide. It makes sense to take a One Health approach to address this problem (McEwen and Collignon 2018). The present study aimed to

provide evidence-based information regarding the occurrence of antibiotic resistance and to determine the virulence factors of *S. aureus* isolates. To the best of our knowledge, this is the first report regarding antibiotic resistance and virulence factors of several isolates of *S. aureus* from dairy farms in South Sulawesi Province, Indonesia.

MATERIALS AND METHODS

Study area

The study was conducted in dairy farms in the Enrekang Regency, South Sulawesi Province, Indonesia from June to August 2021.

Ethical approval

Ethical approval for this study was received from the Health Research Ethics Commission of Hasanuddin University Hospital Number: 105/UN4.6.4.5.31/PP36/2021. Furthermore, before sampling, written informed consent was obtained from all animal owners and human contacts, that participated in this study.

Sample collection

The samples were obtained from humans (skin swab of farmers, n = 20), animals (raw milk, n = 58), dairy products (dangke, n = 14), and the environment (n = 20, clean and wastewater). A random sampling method was used. All samples were stored at 4°C and were sent to the laboratory for bacterial analysis.

Microbiological analysis of *Staphylococcus aureus*

Staphylococcus aureus bacteria were isolated using Baird-Parker Agar (BPA) and then incubated at 37°C for 24-48 hours (Thaker et al. 2012; Vatansever et al. 2016). Bacterial colonies growing on BPA were assessed using Gram staining, catalase test, and coagulase test (Kateete et al. 2010). The *S. aureus* ATCC 25923 strain was used as a positive control. The *nuc* gene target was used to confirm *S. aureus* isolates (Kou et al. 2021). The 16S rRNA gene was used as a housekeeping gene marker (Ogier et al. 2019). A total of 31 *S. aureus* isolates comprising 20 isolates from animals (raw milk sample), 7 from humans (skin swab of farmers), and 4 isolates from dairy products (dangke) were obtained. All isolates were obtained from our previous study that was conducted in May 2021.

Antibiotic sensitivity test

Kirby-Bauer disk diffusion method was used to evaluate antibiotic resistance in *S. aureus* isolates (Akya et al. 2020). The bacterial colonies from nutrient agar (NA) were taken aseptically and diluted using sterile physiological NaCl to achieve the 0.5 McFarland turbidity standard. The suspension was cultured using a sterile spreader on Mueller-Hinton agar (MHA) media and air-dried for 10-15 minutes. Then, the antibiotic disc was placed on the MHA medium, and the plate was incubated at 35°C for 16-18 hours. Antibiotics used consisted of several classes, including ß-lactams (ampicillin, 10 µg/disk; penicillin G, 10 U/disk; oxacillin, 1 µg/disk; cefoxitin, 30 µg/disk), aminoglycosides (gentamicin, 10 µg/disk), tetracyclines (tetracycline, 30 µg/disk), fluoroquinolones (ciprofloxacin, 5 µg/disk), macrolides (erythromycin, 15 µg/disk), and chloramphenicol (chloramphenicol, 30 µg/disk). The antibiotic inhibition zones formed on MHA media were then measured and adjusted to the standards set by CLSI. The standards that have been set include susceptible (S), intermediate (I), and resistant (R) (CLSI 2018).

DNA extraction

Bacteria were extracted from pure cultures using the boiling method (Hassanzadeh et al. 2016) with some modification. First, one loop of bacterial colonies was transferred into 500 µL of TE buffer and then homogenized. Next, 200 µL of bacterial cell suspension was taken and then heated at 95°C for 25-30 minutes and centrifuged at 13000× g for 15 minutes. The pellet was added to 200 µL of nuclease-free water and homogenized.

Identification of antimicrobial resistance genes and virulence factors of *Staphylococcus aureus*

Six antimicrobial resistance genes, including ß-lactam resistance genes (*blaTEM*, *mecA*, *mecC*), tetracycline resistance gene (*tet*A), erythromycin resistance gene (*ermB*), ciprofloxacin resistance gene (*qnrS*), and five virulence factor genes of *S. aureus*, including *fnbA*, *clfA*, *hla*, *sea*, and *tst*, were detected via conventional polymerase chain reaction (PCR), using a specific primer (Table 1). In each PCR run, several strains from our lab were utilized as positive controls for the genes. The PCR analysis was performed in a total volume of 25 µl. The PCR components consisted of 12 µl MyTaq^{TM} Red Mix, 4 µl template, 2 µl forward primer, 2 µl reverse primer, and the total volume was made up to 25 µl using ddh_2O. The PCR was performed in a Thermal Cycler T100^{TM} (Bio-Rad, California, USA) and then visualized using electrophoresis. A total of 5 µL was visualized on 1% agarose gel using 1 µL FloroSafe DNA Stain (1st BASE).

The amplification programs used for the antimicrobial resistance genes were as follows: *blaTEM* gene, 3 min at 95°C, followed by 30 cycles, consisting of 95°C for 15 s, 54°C for 15 s, 72°C for 10 s, and finally, 72°C for 5 min; *tetA* gene, 1 min at 95°C, followed by 30 cycles, consisting of 95°C for 15 s, 62°C for 10 s, 72°C for 10 s, and finally, 72°C for 10 s; *ermB* gene, 5 min at 95°C, followed by 30 cycles, consisting of 95°C for 30 s, 54°C for 1 min, 72°C for 1 min, and finally, 72°C for 8 min; *mec*A gene, 5 min at 94°C, followed by 30 cycles, consisting of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and finally, 72°C for 1 min; *mec*C gene, 3 min at 95°C, followed by 30 cycles, consisting of 95°C for 35 s, 59°C for 1 min, 72°C for 1 min, and finally, 72°C for 7 min; and *qnrS* gene, 3 min at 95°C, followed by 30 cycles, consisting of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min, ending with a final extension step at 72°C for 5 min.

Table 1. Primers used in this study for PCR amplification

Target gene	Primer name	The nucleotide sequence (5′-3′)	Product size (bp)	References
16S rRNA	Bac-F1	ACAGTTTGATCMTGGCTCAG	1500	Kepel and Fatimawali (2015)
	Uni-B1	GGTTACSTTGTTACGACTT		
nuc	nuc-F	GCGATTGATGGTGATACGGTT	270	Kou et al. (2021)
	nuc-R	AGCCAAGCCTTGACGAACTAAAGC		
blaTEM	TEM-C	ATCAGCAATAAACCAGC	516	Abrar et al. (2019)
	TEM-H	CCCCGAAGAACGTTTTC		
tetA	tet(A)-F	GGTTCACTCGAACGACGTCA	577	Indrawati et al. (2021)
	tet (A)-R	CTGTCCGACAAGTTGCATGA		
mecA	mecA-1	GGGATCATAGCGTCATTATTC	162	Tsai et al. (2017)
	mecA-2	AACGATTGTGACACGATAGCC		
mecC	mecC-F	TGTTGTAGCAATGTTCACAC	138	Gergova et al. (2019)
	mecC-R	CAAGCACTTAATATCAACGC		
ermB	ErmB-F	GAAAAGGTACTCAACCAAATA	639	Flórez et al. (2014)
	ErmB-R	AGTAACGGTACTTAAATTGTTTAC		
qnrS	qnrSF	ACGACATTCGTCAACTGCAA	417	Mahmud et al. (2018)
	qnrSR	TAAATTGGCACCCTGTAGGC		
fnbA	fnbA_F	ACT TCA CCT GTC GCC ATT AC	539	Acosta et al. (2018)
	fnbA_R	GCA GTA CAA GCA CCA CAA AC		
*clf*A	clfA_F	GAT TCT GAC CCA GGT TCA GA	945	Acosta et al. (2018)
	clfA_R	CTG TAT CTG GTA ATG GTT CTT T		
hla	hla_F	GGT TTA GCC TGG CCT TC	534	Salasia et al. (2011)
	hla_R	CAT CAC GAA CTC GTT CG		
sea	GSEAR-1	GGTTATCAATGTGCGGGTGG	102	Rossato et al. (2018)
	GSEAR-2	CGGCACTTTT TTCTCTTCGG		
tst	GTSSTR-1	ACCCCTGTTCCCTTATCATC	326	Rossato et al. (2018)
	GTSSTR-2	TTTTCAGTATTTGTAACGCC		

The amplification programs used for virulence factor genes were as follows: *clfA* and *hla* genes, 5 min at 94°C, followed by 30 cycles, consisting of 94°C for 30 s, 57°C for 30 s, 72°C for 30 s, and a final extension step at 72°C for 10 min; *fnbA* gene, 5 min at 94°C, followed by 30 cycles, consisting of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s, and finally, 72°C for 10 min; *tst gene*, 5 min at 94°C, followed by 30 cycles, consisting of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension step at 72°C for 10 min; and *sea* gene, 5 min at 94°C, followed by 30 cycles, consisting of 94°C for 30 s, 54°C for 30 s, 72°C for 30 s, and a final extension step at 72°C for 10 min.

Data analysis

The results of the bacterial susceptibility test, antibiotic resistance gene, and virulence factors of *S. aureus* are shown in the form of tables and figures and were analyzed descriptively.

RESULTS AND DISCUSSION

Antibiotic resistance

Staphylococcus aureus isolated from raw milk exhibited resistance to penicillin G (50%), ampicillin (50%), tetracycline (15%), erythromycin (5%). The human *S. aureus* isolates showed resistance to penicillin G (86%), ampicillin (86%), oxacillin (14%), cefoxitin (14%), tetracycline (43%), and ciprofloxacin (14%). Meanwhile, isolates from dairy products (dangke) showed resistance to antibiotics penicillin G (50%) and ampicillin (50%) (Table 2, Figure 1).

The highest frequency of resistance to antibiotics in animal isolates (raw milk) was from the β-lactam group of antibiotics. This finding is in line with the results of previous research in Surabaya, Indonesia (Tyasningsih et al. 2019). A study by Mbindyo et al. (2021) reported the frequency of resistance to antibiotics ampicillin (71.4%), tetracycline (21%), erythromycin (25.2%) in Kenya. Meanwhile, a previous study by Liu et al 2017 reported the incidence of resistance to antibiotics penicillin G (85.2%), ampicillin (79.6%), tetracycline (13%), and erythromycin (46.3%) in China. The high level of resistance in this study could be attributed to the widespread use of β-lactam antibiotics to treat mastitis in dairy cows (Fejzic et al. 2014). In Indonesia, as in many other developing nations, most of these antibiotics are inexpensive and readily available as over-the-counter medications that can be purchased without a veterinarian prescription (Majalija et al. 2020). High-frequency resistance rates to penicillin, tetracycline and ciprofloxacin have been observed in human isolates (Ragbetli et al. 2016; Derakhshan et al. 2021). Overuse and misuse of antibiotics in humans have contributed greatly to antimicrobial resistance (Byrne et al. 2019).

Table 2. Profile of antimicrobial resistance of *Staphylococcus aureus* isolates

No	Code	PEN	AMP	OXA	FOX	CN	TE	CIP	E	C
Animal isolates										
1	S10	S	S	S	S	S	S	S	S	S
2	S12	S	S	S	S	S	S	S	S	S
3	S14	R	R	S	S	S	R	S	S	S
4	S15	R	R	S	S	S	S	S	S	S
5	S16	R	R	S	S	S	S	S	S	S
6	S18	S	S	S	S	S	S	S	R	S
7	S21	S	S	S	S	S	S	S	S	S
8	S22	S	S	S	S	S	S	S	S	S
9	S24	R	R	S	S	S	S	S	I	S
10	S26	R	R	S	S	S	S	S	I	S
11	S28	S	S	S	S	S	S	S	S	S
12	S29	S	S	S	S	S	S	S	S	S
13	S30	R	R	S	S	S	R	S	S	S
14	S31	R	R	S	S	S	R	S	S	S
15	S36	S	S	S	S	S	S	S	S	S
16	S37	R	R	S	S	S	S	S	S	S
17	S39	R	R	S	S	S	S	S	S	S
18	S41	R	R	S	S	S	S	S	S	S
19	S42	S	S	S	S	S	S	S	S	S
20	S43	S	S	S	S	S	S	S	S	S
n = 20		10	10	0	0	0	3	0	1	0
		50%	50%	0	0	0	15%	0	5%	0
Human isolates										
1	H1	R	R	S	S	S	S	S	S	S
2	H2	R	R	S	S	S	S	S	S	S
3	H3	S	S	S	S	S	S	S	S	S
4	H4	R	R	S	S	S	S	S	S	S
5	H5	R	R	R	R	S	R	R	S	S
6	H6	R	R	S	S	S	R	S	S	S
7	H7	R	R	S	S	S	R	S	I	S
n = 7		6	6	1	1	0	3	1	0	0
		86%	86%	14%	14%	0	43%	14%	0	0
Dairy product isolates (*dangke*)										
1	D01	R	R	S	S	S	S	S	S	S
2	D05	R	R	S	S	S	S	S	S	S
3	D10	S	S	S	S	S	S	S	S	S
4	D12	S	S	S	S	S	S	S	S	S
n = 4		2	2	0	0	0	0	0	0	0
		50%	50%	0	0	0	0	0	0	0

Note: R: resistance; I: Intermediate; S: Susceptible; PEN: penicillin G; AMP: ampicillin; OXA: oxacillin; FOX: cefoxitin; CN: gentamicin; TE: tetracycline; CIP: ciprofloxacin; E: erythromycin; C: chloramphenicol.

Antibiotic resistance marker gene

Nineteen isolates of *S. aureus* were selected to detect antimicrobial resistance gene (ARGs) involved in the resistance to ß-lactams, tetracycline, macrolide, and ciprofloxacin, namely *blaTEM*, *mecA*, *mecC*, *tetA*, *ermB*, and *qnrS*. Table 3 presents the ARGs detected in animal, human, and dairy product isolates and their antibiotic resistance profiles. The frequency of ARGs from animal isolates (raw milk) is *blaTEM* (90%) and *tetA* (100%). These isolates did not harbor the *mecA*, *mecC*, and *ermB* genes. The *bla*TEM (83%), *mec*A (17%), and *tet*A (100%) were detected in the isolates from human samples, but *mecC* and *qnrS* were not detected. Simultaneously, the *blaTEM* gene (100%) was detected in dairy product isolates (*dangke*), but *mecA* and *mecC* were not detected.

The *blaTEM* was detected in most isolates, which was positively correlated with the penicillin G resistance phenotype. Previously, studies reported a high frequency of the *bla*TEM gene in foodborne and clinical isolates (80% and 100%, respectively) (Xu et al. 2014; Yang et al. 2016). Resistance to methicillin in *S. aureus* is primarily mediated by the *mec*A gene, which encodes the low-affinity penicillin-binding protein 2a (PBP2a) (Rolo et al. 2017). *Staphylococcus aureus* isolates from animals (raw milk) did not harbor *mecA* and *mecC*, similar to the findings of a previous study (Andrade et al. 2021). In contrast, Parco et al. (2021) reported the isolation of *S. aureus* strains from milk samples that harbor *mecA*. In this study, the *mecA* gene was detected in *S. aureus* from a human sample. The presence of *mecA* in an *S. aureus* strain from a human sample has been reported previously by other authors (Wielders et al. 2002). The presence of the tetA gene (11.8%) in food and clinical isolates (8.3%) is associated with the tetracycline resistance phenotype (Arab et al.

2018; Ma et al. 2018). This study did not report the presence of the *ermB* gene from the animal samples (raw milk) or the *qnrS* gene from human samples (skin swab of the farmer). Contrastingly, previous studies reported the presence of the antibiotic resistance genes *erm*B and *qnr*S from animal and human samples, respectively (Abdu and Mirabeau 2019; Mbindyo et al. 2021). There is a discrepancy between ARGs and phenotypic confirmation. It is possible that the antibiotic itself modulates the levels of ARGs, resulting in their low *in vitro* expression, or that the heteroresistance phenomenon is associated with rare mutations, unstable tandem gene amplification, and environmental modulation of the resistance genes explains the observed differences in susceptibility. A comprehensive whole-genome sequencing (WGS)-based investigation can be used to identify accurate genotype-to-phenotype resistance associations (Urmi et al. 2020).

The presence of antimicrobial resistance genes in *S. aureus* from animals, humans, and dairy products (dangke) is a public health concern. The ARGs in raw milk can be transmitted from non-pathogens to pathogens via horizontal gene transfer (HGT), resulting in the rapid establishment of multidrug resistance (MDR) in bacteria from animals, posing a foodborne risk to human health. The presence of AGRs in unprocessed animal products may contribute to the development of antimicrobial resistance in human pathogens (McMillan et al. 2019; Toth et al. 2020). ARGs can spread among animals, farms, various other environments, agriculture, and the human community and households (Graham et al. 2019).

Virulence marker genes

Nineteen isolates were selected for the detection of virulence factor genes in *S. aureus*. The frequencies of virulence genes detected in this study were as follows (in *S. aureus* isolates derived animal and human samples): *fnb*A (36% and 17%, respectively), *hla* (45% and 67%, respectively), *clf*A (45% and 67%, respectively), and *tst* (18% and 33%, respectively); the *sea* gene was not detected in isolates from the animal (raw milk), and human (skin swab of farmers) samples. While the *hla* gene (100%) and *clf*A gene (100%) were detected in the dairy product (dangke), the *fnb*A, *tst*, and *sea* gene were not detected (Table 3, Figure 2).

The pathogenicity of *S. aureus* is related to its ability to produce and secrete a variety of virulence factors that play a role in the colonization, invasion, and degradation of host cells, immune evasion, and bacterial spread (Pietrocola et al. 2017; Cheung et al. 2021). The *fnb*A and *clf*A genes are virulence factors implicated in host cell adhesion, while the *sea*, *tst*, and *hla* were reported to be associated with toxins (Acosta et al. 2018). The present study revealed that virulence factors *fnb*A, hla, *clf*A, and *tst* genes were detected in *S. aureus* isolates derived from animal and human samples. Meanwhile, isolates from dairy products (dangke) showed the presence of *hla* and *clf*A. None of the *S. aureus* isolates obtained in the present study harbored the *sea* gene. The existence of *fnb*A, *clf*A, *hla*, *sea*, and *tst* in isolates from animal samples has been previously reported by Acosta et al. (2018). Another study reported the presence of the *tst* gene, but not the *sea* gene in the isolates from animal (raw milk) and human samples (Vitale et al. 2019). In contrast, previous findings have reported the presence of the *sea* in human isolates (Saadati et al. 2011). The *sea* gene is the most commonly associated source of foodborne poisoning in humans due to improper handling and storage (Chen et al. 2018) and cases of subclinical mastitis in cattle (Fursova et al. 2018). The toxic shock syndrome toxin-1 (TSST-1), which is encoded by the *tst* gene, is a significant virulence factor in *S. aureus* infections which can trigger excessive and non-conventional T-cell activation and cytokines release and consequently interfere with the immune system function systemically (Zhao et al. 2019). Moreover, the *tst* gene can cause food poisoning in humans (Pérez et al. 2020). *Staphylococcus aureus* produces α haemolysin (*α*-toxin), encoded by the *hla* gene, which contributes to bacterial invasion and escapes from the host immune response (Divyakolu et al. 2019). The *fnb*A and *clfA* genes play a role in host cell invasion and evasion of the host immune responses (Stutz et al. 2011).

Several studies have been reported the relationship between the presence of ARGs and virulence factors in bacteria (Schroeder et al. 2017; Cepas and Soto 2020; Pan et al. 2020; Pérez et al. 2020). The expression of ARGs can be influenced by the regulation of virulence gene expression and vice versa. Various environmental factors influence the regulation of these genes, either directly or indirectly (Schroeder et al. 2017). The relationship between virulence factors and antimicrobial resistance depends on bacterial species, the specific mechanism underlying antimicrobial resistance, the ecological or environmental niche, and the host (the immune system) (Beceiro et al. 2013).

In conclusion, this study revealed that *S. aureus* isolates from humans, animals, and dairy products (dangke) were resistant to several types of antibiotics. The *blaTEM* gene, which was present in the isolates from humans, animals, and dairy products (dangke), has the potential to spread between isolates and poses a potential human health risk. Therefore, the proper use of antibiotics in humans and animals needs to be implemented in local communities to prevent the spread of antibiotic resistance. The presence of *tst* in raw milk is a very important issue that must be considered to protect consumers against the risk of toxic shock syndrome. Therefore, it is necessary to pay attention to the handling and processing of dairy products to ensure their safety for human consumption.

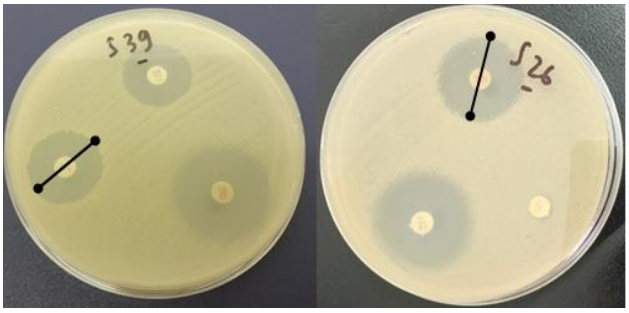

Figure 1. Diameter of the growth-inhibition zone obtained using the disc diffusion method (black line)

Table 3. 16S rRNA gene profiles, antimicrobial resistance genes, and virulence factors of *Staphylococcus aureus*

No	Code	16S rRNA	Antimicrobial resistance gene						Virulence factor of *Staphylococcus aureus*				
			*bla*TEM	*mec*A	*mec*C	*tet*A	*erm*B	*qnr*S	*fnb*A	*hla*	*clf*A	*sea*	*tst*
Animal isolates													
1	S14	+	+	-	-	+	nd	nd	-	-	-	-	-
2	S15	+	+	-	-	nd	nd	nd	-	-	-	-	-
3	S16	+	+	-	-	nd	nd	nd	-	-	-	-	-
4	S18	+	nd	nd	nd	nd	-	nd	-	-	-	-	-
5	S24	+	+	-	-	nd	-	nd	-	-	-	-	-
6	S26	+	+	-	-	nd	-	nd	-	-	-	-	-
7	S30	+	+	-	-	+	nd	nd	+	+	+	-	-
8	S31	+	-	-	-	+	nd	nd	+	+	+	-	-
9	S37	+	+	-	-	nd	nd	nd	+	+	+	-	+
10	S39	+	+	-	-	nd	nd	nd	+	+	+	-	+
11	S41	+	+	-	-	nd	nd	nd	-	+	+	-	-
n=11		11/11	9/10	0	0	3/3	0	0	4/11	5/11	5/11	0	2/11
		100%	90%	0	0	100%	0	0	36%	45%	45%	0	18%
Human isolates													
1	H1	+	-	-	-	nd	nd	nd	-	+	+	-	-
2	H2	+	+	-	-	nd	nd	nd	-	+	+	-	+
3	H4	+	+	-	-	nd	nd	nd	-	+	+	-	-
4	H5	+	+	+	-	+	nd	-	-	-	-	-	-
5	H6	+	+	-	-	+	nd	nd	+	+	+	-	+
6	H7	+	+	-	-	+	nd	nd	-	-	-	-	-
n=6		6/6	5/6	1/6	0	3/3	0	0	1/6	4/6	4/6	0	2/6
		100%	83%	17%	0	100%	0	0	17%	67%	67%	0	33%
Dairy product isolates													
1	D01	+	+	-	-	nd	nd	nd	-	+	+	-	-
2	D05	+	+	-	-	nd	nd	nd	-	+	+	-	-
n=2		2/2	2/2	0	0	0	0	0	0	2/2	2/2	0	0
		100%	100%	0	0	0	0	0	0	100%	100%	0	0

Note: -: negative; +: positive; nd: not detected; n: number of isolates.

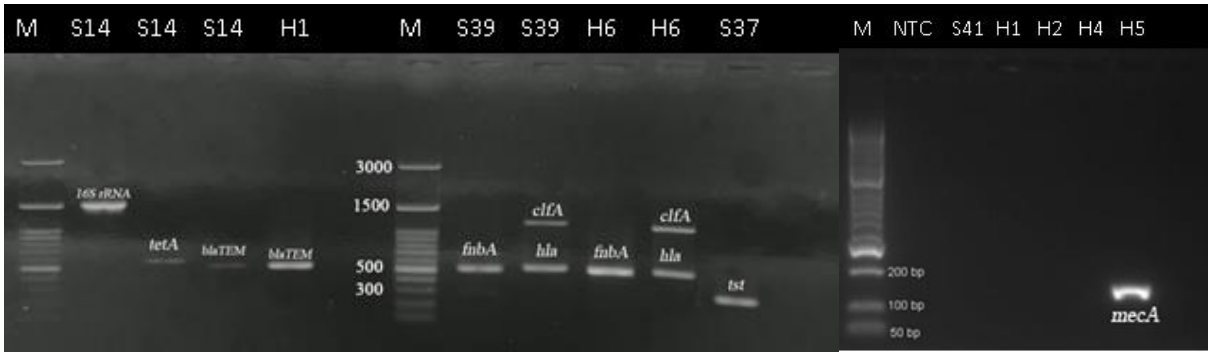

Figure 2. PCR products obtained after the amplification of the *16S rRNA* gene (1500 bp), antimicrobial resistance genes, including *tet*A (577 bp), *blaTEM* (516 bp), and *mecA* (162 bp), and virulence factors of the *Staphylococcus aureus* isolates, including *fnbA* (539 bp), *hla* (534 bp), *clfA* (945 bp), and *tst* (326 bp)

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