https://doi.org/10.26682/sjuod.2023.26.2.16
Journal of University of Duhok., Vol. 26, No.2 (Pure and Engineering Sciences),Pp 199-206, 2023

PREVALENCE OF VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM DAIRY PRODUCTS IN KURDISTAN REGION-IRAQ

HAVAL **A**BDULLAH **K**HUDHER[*], **Z**AINAB **M. A**LZUBAIDY[**] and **J**ASEM **M**. **A**BDO[***]
[*]Dept. of Biology, Faculty of Science, Soran University, Kurdistan Region-Iraq
[**]Dept. of Biology, College of Sciences, Diyala University-Iraq
[***]Dept. of Clinical Pharmacy, college of Pharmacy, University of Duhok, Kurdistan Region-Iraq

(Received: August 6, 2023; Accepted for Publication: September 11, 2023)

ABSTRACT

The context highlights the importance of *Staphylococcus aureus* as a potential cause of foodborne illnesses, its capacity to become resistant to antibiotics such as vancomycin, and the possible dangers posed by dairy products contaminated with VRSA to public wellbeing. The main aim of the research is to gauge the prevalence of VRSA in dairy items within the Kurdistan Region, revealing potential risks to both food security and the well-being of consumers. So, in this study, the resistance of 69 of *S. aureus* bacteria isolated from 300 Kurdish locally cheese, hard cheese, soft cheese, yogurt, samples to six types of antibiotics (clindamycin, tetracycline, Rifampicin, erythromycin, vancomycin and Ampicillin) were evaluated. The 69 isolates were identified by Vitec system2, also confirmed by specific primer of 16SrRNA gene, those isolated both results are the same. Antimicrobial resistance of strains to six antibiotics was determined by using Vitec system2, and disc diffusion method. The antibacterial resistance among all the isolated were detected against Erythromycin 53(77%), clindamycin 65(94), vancomycin 25(36%), tetracycline 62(90%), Rifampicin 61(88%), ampicillin 69(100%). Antibiotic resistance genes were studied by polymer chain reaction and the following genes were detected, in totally 19 isolated present Van (A), and 16 isolated present Van(B) from 25 isolated they were resistance to vancomycin as antibacterial.

.***KEY WORDS:*** Antibiotics, Resistance, Vancomycin, Bacteria, Dairy Product.

INTRODUCTION

The germ *S. aureus* is opportunist a pathogenic bacterium that has been documented for its tendency to invasive diseases and cause serious. Usually arranged in grape-like irregular clusters(Becker, *et al*. 2015). It convex, lustrous, creates smooth and circular colonies, in necessity on development condition, the colony coloring varies from grey, grey-white with yellowish to oranges. The disease-causing *S. aureus* formed a golden yellow dye, it is an opportunistic bacterium frequently part of human microflora, it is a number of *Staphylococcaceae,* a taxonomic group that contains thirty tree other members based on 16SrRNA sequencing, *Staphylococcaceae* is taxonomically placed between *Bacilliaceae* and *listeriaceae*(Patel, *et al*. 2018, Yuan, *et al.* 2020).

Food-borne infection defined by World Health Organization (WHO), as infections, commonly food born disease toxic infection, that have two types of illness, food born infection and food born intoxication, produced via agents that arrived the body through the digestion of dairy products or another types of food(Ishaq, *et al*. 2021). Microbial contamination of dairy products characterizes one of the main community significant difficulties, the most important bacteria species that cause community health problems worldwide is *S. aureus* (Hernández-Cortez, *et al*. 2017).

Food-borne illnesses are main difficulties, mainly in developing countries, and cause the common of infections, and death about the world. Dairy products are the most important vehicle that transmits the pathogenic bacteria to the body of human(Thomas, *et al.* 2016, Abebe, *et al.* 2020). Universal, food-borne syndromes are a most important health load leading to high mortality and morbidity. The worldwide problem of infectious diarrhea includes 3-5 billion cases and closely 1.8 million deaths yearly, mostly in children, caused by polluted

dairy products (food) and water(Abebe, Gugsa et al. 2020).

Drug resistance or antibiotics resistance can be defined as a microorganism or bacteria have ability to survive, and grow when treated by antimicrobial. Many strains of bacteria become resistance to one or more types of antibacterial. This phenomenon is named multidrug resistance(Davies and Davies 2010). Antibiotic resistance is a global problem. New form of antimicrobial resistance can cross international borders and spread effortlessly between the continents(Dugassa, *et al.* 2017). Many forms of resistance spread with it wonderful speed. World health leaders describe antibiotics resistant bacteria as "nightmare bacteria" that "represent catastrophic threat" for individuals in all nations of the world. Annually around the word, as a minimum four million people get thoughtful infections with germ that are resistant to one or more of the antimicrobial striped to treat those infections Most infection causing bacteria are resistant to at least one of the antimicrobial that are normally used to destroy the infection(Dizaj, *et al*. 2015, Dugassa and Shukuri 2017). The increasing resistance of the microbes to antimicrobial has been led to serious health problems in the recent years. The problem of microbial resistance to antibiotics prompted researchers to work on creating an antibiotic that prevents the growth of bacteria, the current study aimed to isolate *S. aureus* from dairy products and determine their antibiotics sensitivity in Kurdistan Region.

METHODOLOGY

Collection and Isolation Method:

Three hundred (300) local dairy products samples; from October, 2021 to January 2022, were collected from local markets in Kurdistan region (Table No.1), each sample was sited in dispersed sterile plastic container, aseptically carried under refrigeration condition to laboratory and analyzed. Twenty-five grams of each sample were suspended in 225ml of normal saline. Serial decimal dilution was prepared from this initial dilution, each of the different suitable dilution (1ml.) was transferred onto surface of mannitol salt agar that was commonly used as a screening media for *S. aureus* and incubated at 35^{o}C for 48 hrs. (Boukharouba 2022); (Mushore and Matuvhunye 2013). Also used some biochemical test to confirm this bacterium, gram staining, mannitol fermentation, catalase, lipase, β- Hemolysis, coagulases, oxidases, (Table No.2).

Genomic DNA Extraction from Isolated Bacteria:

DNA was extracted from the suspected *S. aureus* colonies using High Pure PCR Template Preparation Kit (mericon™ DNA Bacteria Handbook-Germany) according to the manufacturer's instructions. (we added 400 µl Fast Lysis Buffer to the bacterial pellet, tightly cap the tube, and resuspend the pellet by brief, vigorous vertexing. The microcentrifuge tube was placed into a heating block or thermal shaker (800 rpm) at 100°C. for 10 min. samples were removed and allowed, to cooldown to room temperature (15–25°C) for 2 min. The tube centrifuge at 13,000 rpm. for 5 min. Transfer 100 µl of the supernatant to a fresh 1.5 ml microcentrifuge tube. The aliquot of the collected supernatant was used directly in a PCR reaction. The remaining supernatant was discarded, unless testing for the presence of viable bacteria).

Antimicrobial Susceptibility Test

All isolated *S. aureus* were identified and confirmed, then inoculated on brain heart infusion broth, after being tested for antibacterial susceptibility using the Kirby-Bauer disk diffusion technique and interpreted in accordance with the (CLSI) rules. The suspension from the tube that had been incubated at 37°C for 24 hours was then spread equally on the Mueller Hinton agar surface culture media. The disc agar method, which has been adopted as industry standard practice for determining antibiotic susceptibilities, was used to evaluate (NCCLS, 2020). (Humphries, *et al.* 2021). verified by the Vitec systems tool as well.

Detection of 16srRNA of Bacteria Genes and Van A, Van B genes by PCR:

The extracted bacteria-specific DNA fragment sequence gene's primer (F ¯5GTAGGTGGCAAGCGTTATCC3¯ , R ¯5 CGCACATCAGCGTCAG3¯). The Ready MixTMTaq PCR Reaction Mix with MgCl2, Sigma, 12.5 µL PCR premix, 0.4 M of each primer, and 2 µL DNA sample were used to create a PCR reaction mix (25 µL). The following settings were used for DNA amplification using a thermal cycler (Mastercycler Personal, Eppendorf): initial denaturation for 2 min at 94°C, followed by 40 cycles of denaturation at 94°C for 20 sec, annealing at 56°C for 30 sec, and extension at 72°C for 30 sec. With some variation in

annealing and extension temperatures for each variety of bacteria, the final extension was performed at 72°C for 5 min. The product sizes for VanA (F 5′GGG AAAACGACAATTGC3, R 5′GTACAATGCG GCCGTTA-3); for VanB, are (F 5′-ATGGGAAGCCGATAGTC-3, and F 5′-GATTTCGTTCCTCGA CC-3). Respectively (Praharaj, Sujatha et al. 2013). The PCR procedure involved a pre-denaturation phase at 94°C for 5 minutes, followed by 30 cycles of 45 seconds each at 94°C, 59°C, and 72°C (Al-Kalabi, *et al*. 2021). The final extension stage was carried out at 72°C for 5 minutes. The concentration of agarose in a gel is typically between 0.5% and 2%, depending on the sizes of the DNA pieces to be separated. A casting tray is used as a mold after the solution has been put into it and cooled to about 55 0C. To create wells when the gel solution solidified, a well-former template (commonly referred to as a comb) is laid across the edge of the casting tray.

Results

Isolation and Identification of *Staphylococcus aureus*

A total of 300 dairy product samples were taken from different places Kurdistan region areas (Duhok, Hawler and Sulaimania) to examined pathogenic bacteria, as appeared in table (1), the samples included; cheese, soft cheese, hard cheese and yogurt (75) samples for each one. Out of these food samples, 195(65%) of the total samples were contaminated with bacteria. According to the results the highest contaminated food was cheese (84%), while the soft cheese (75%), hard cheese was (70%) and (52%) of yogurt was contaminated with pathogenic bacteria, but totally only 65% of dairy products are contaminated by *S. aureus*.

Table (1): Isolation and Identification of *Staphylococcus aureus*

Type of dairy product	Total no. of examined samples	No. of positive samples on M.S. A	percentage of positive sample on M.S. A	No. and percentage of positive *S.aureus*
Cheese	**75**	**60**	**84%**	**(15) 20%**
Hard cheese	**75**	**46**	**70%**	**(10) 13%**
Soft cheese	**75**	**56**	**75%**	**(20) 17%**
Yogurt	**75**	**33**	**52%**	**(15) 20%**
Totally	**300**	**195**	**65%**	**(60) 20%**

Physiological characters of *Staphylococcus aureus*

Mannitol salt agar media which is considered as a selective and differential media to identify the genus of *Staphylococci*, showing the colonies of *S. aureus* round, smooth, raised, mucoid and glistening. Sample were positive for *S. aureus* that grew on mannitol forming large golden colonies surrounded by wide yellow zones and changed the color of the medium from pink to yellow in addition biochemical tests were conducted on the samples as a confirmation for the contaminated foods with *S. aureus*, the characters of 69 isolates of the bacteria which obtained from the total food samples were determined as appeared in Table 2.

Table (2): Physiological characters of *Staphylococcus aureus* isolates

Tests	No. and percentage of positive isolates	
	No. of positive isolates	%
Mannitol fermentation	**58**	**84%**
β- Hemolysis	**55**	**79%**
+ Gram stain	**69**	**100%**
Catalase	**69**	**100%**
Oxidase	**3**	**4%**
Lipase	**52**	**75%**
Coagulase	**69**	**100%**

Identification of *Staphylococcus aureus* by PCR

Total of 69 isolates of *S. aureus* which identified by biochemical tests, and VITEK 2 system tool from the total isolates; 69 isolates from different locally sources of dairy product as follow: (15) from cheese and (11) from hard cheese, (20) from soft cheese, and (14) from yogurt samples. Later on, the rapid method by PCR was also done, for the 69 samples, as confirmation test, and the results showed that 87% of the 69 samples, gave positive result to specific sequence 16rRNA gene as shown in Table 3.

Table (3): Detection *Staphylococcus aureus* by PCR Technique.

Type of source	No. of isolates identified by biochemical tests	No. and % of positive isolates by PCR
cheese	18	15(83%)
hard cheese	12	11(92%)
soft cheese	22	20(90%)
Yogurt	17	14(82%)
Total	69	60(87%)

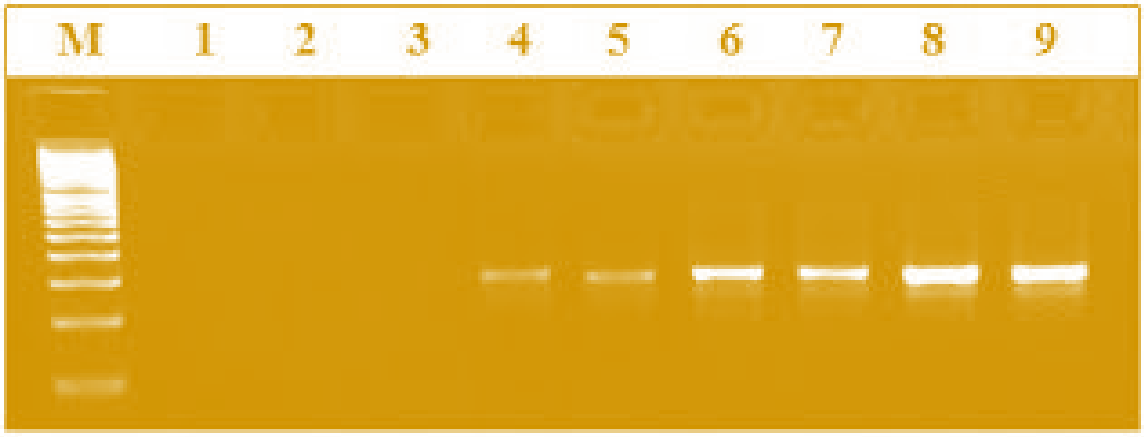

Fig. (1): Identification of *Staphylococcus aureus* by PCR (amplification of 16srRNA gene product size 228bp). Lane M is 285bp ladder, S4 to S9 positive amplification for 16srRNA gene, S1 and S3 negative amplification for 16srRNA gene.

Antibiotic susceptibility tests

The antimicrobial susceptibility test was performed manually according to Kirby Bauer method (Antibiotic disc diffusion method) with most types of antibiotics, were applied against all isolated of *S. aureus*, as well as determination minimum inhibition concentration. The test was applied to find the multidrug resistance pattern that was usually associated with resistance of these bacteria and to find the most effective therapy. So, using the VITEC2 System to conforming susceptibility, as shown in Table 4.

Table (4). Detection of Antibiotic susceptibility tests to *Staphylococcus aureus*.

Antimicrobial	MIC	Susceptibility	No. of sample	% Resistance
Cefoxitin	Positive	<<	69	100%
Benzylpenicillin	>=0.5	<<	69	100%
Oxacillin	>=4	<<	69	100%
Gentamicin	>=0.5	S	69	100%
Tobramycin	>=2	S	69	100%
Levofloxacin	1	S	69	100%
Moxifloxacin	>=0.25	S	69	100%

Inducible clindamycin	Negative	-	69	100%
Erythromycin	1	I	53	77%
Clindamycin	>=8	R	65	94%
Linezolid	2	S	69	100%
Teicoplanin	>=0.5	S	69	100%
Vancomycin	>=32	R	25	36%
Tetracycline	>=16	R	62	90%
Tigecycline	<=0.12	S	69	100%
Fosfomycin			69	100%
Nitrofurantoin	32	S	69	100%
Fusidic acid			69	100%
Mupirocin			69	100%
Rifampicin	8	R	61	88%
Trimethoprim/ sulfamethoxazole	<=10	S	69	100%
Ampicillin		R	69	100%

Detection of antibiotics resistance gene in *staphylococcus aureus* isolates

The table provides information about the presence of vancomycin-resistant *S. aureus* in different dairy products, as well as the proportion of the specific vancomycin resistance genes, Van A and Van B, within these bacteria. The dairy items listed include "Cheese," "Hard cheese," "Soft cheese," and "Yogurt." Within these categories, "Cheese" has 7 *S. aureus* bacteria displaying vancomycin resistance, "Hard cheese" contains 3 such bacteria, "Soft cheese" exhibits 8 resistant bacteria, and "Yogurt" has 7. The overall count of vancomycin-resistant bacteria across all dairy products is 25. Additionally, the table highlights that 36% of the resistance genes are identified as Van A, while 32% are categorized as Van B. These specific genes are known to be significant in bestowing resistance to vancomycin in *S. aureus*, underscoring the importance of monitoring antibiotic resistance in dairy products to address public health concerns.

Table (5): Detection of antibiotics resistance gene in *staphylococcus aureus* isolates

	No. of *Staphylococcus aureus* resistance to vancomycin					
	Cheese	Hard cheese	Soft cheese	Yogurt	Totally	% Of gene present
Staph. Aureus	7	3	8	7	25	
Van A	3	1	3	2	09	36%
Van B	2	2	2	2	08	32%

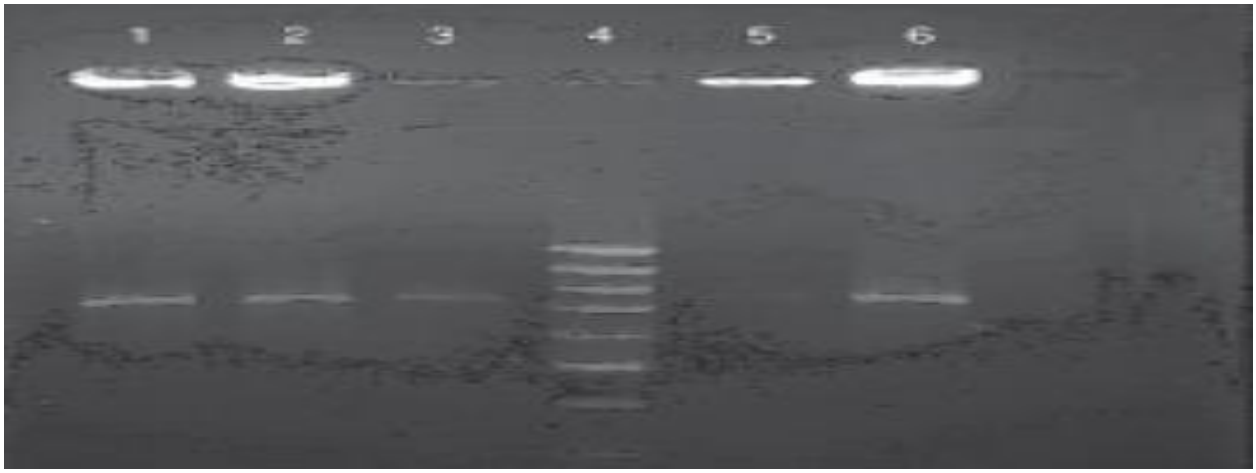

Fig. (2): Identification resistance genes Van(A) and Van(B) of *Staphylococcus aureus* by PCR (amplification of 16srRNA gene product size 228bp). Lane M is (1000-285bp) ladder, S1 to S3 and S6 positive amplification for 16srRNA gene, S5 negative amplification for 16srRNA gene.

DISCUSSION

The table 1 provides insight into the prevalence of *S. aureus* in different types of dairy products. Notably, the percentage of positive samples on M.S. A varies across the categories, with Cheese having the highest (84%) and Yogurt having the lowest (52%). However, when it comes to the actual presence of *S. aureus*, Yogurt shows the highest percentage (20%) among the positive samples on M.S. A, while Hard cheese has the lowest (13%). These variations highlight the need for specific control measures and quality assurance in dairy product production to ensure consumer safety and health. Further analysis and research may be necessary to understand the factors contributing to these differences in prevalence among the dairy product types. Different kinds of microorganisms can contaminate dairy products coming from a variety of sources, including air, dirt, sewage, water, feeds, people, food ingredients, tools, packages, and insects. Depending on the level of sanitation used when handling foods, microbial types and their amounts from these sources can differ greatly(Uyttendaele, *et al.* 2015). The dairy product samples were taken and examined for *Staphylococcus* along with *S. aureus* which cultivated on specific selective media, 20% of the total samples were contaminated with *S. aureus.* All isolates passed the catalase test, which distinguishes the genus *Staphylococcus* from the genus *Streptococcus*, that produced negative (Alakbaree, *et* al. 2022).The majority of the isolates failed the Oxidase test used to distinguish *Staphylococcus* from the typically positive Micrococcus species (Becker, *et al.* 2015). All of the *S. aureus* isolates that were subjected to the coagulase test for additional confirmation produced a positive reaction, which is consistent with the findings of Manukumar and Umesha (2017). To distinguish *S. aureus* from other staphylococci, the ability to ferment mannitol sugar anaerobically was tested. The results showed that 84% of the isolated bacteria had this ability. These findings were similar to those of Oliveira, Costa et al. (2020), who found that 73% of *S. aureus* isolated from cheese samples were able to ferment mannitol, and Elbehiry, Al-Dubaib et al. (2016), who evaluated 76.92% of *S. aureus*.

The data presented in Table 3 highlights the efficacy of the Polymerase Chain Reaction (PCR) method in the identification of *S. aureus* within dairy products. It is noteworthy that Hard cheese demonstrated the highest PCR detection rate, reaching 92%, closely followed by soft cheese with a detection rate of 90%. In contrast, Cheese and Yogurt exhibited somewhat lower but still statistically significant detection rates of 83% and 82%, respectively. This collective information underscores the dependability of PCR as a robust tool for detecting *S. aureus* across diverse dairy product categories. These findings are of paramount importance in the context of food safety and quality control initiatives, as they offer valuable insights into the presence of this pathogenic microorganism in dairy items and can provide guidance for the development of strategies aimed at mitigating the health risks associated with its consumption. To gain a more comprehensive understanding of the factors influencing the variability in detection rates among distinct dairy product groups, further research may be warranted.

S. aureus possessed specific gene 16rRNA gene size (228bp) cleared as bands on gel electrophoresis as shown in Figure1. These results were consistent with the result obtained by Lu, Chen et al. (2023). They by using PCR, detected 87 % of the different dairy product sources, positive for the presences of *S. aureus* and 13% of the different dairy product sources are negative. One of the most prevalent pathogens among the people is *S. aureus,* which is thought to colonize about 25% of healthy adults. Enterotoxins are produced by a variety of pathogenic types and, when consumed, can result in gastroenteritis. In these *S. aureus* enterotoxin outbreaks, *S. aureus* intoxication has been linked to both suspected and proven foodborne outbreaks that have been reported in chicken, beef, seafood, lamb, pasta salad, and rice dishes (Bintsis, 2017).

The table 4 offers valuable insights into the susceptibility patterns of *S. aureus* isolates to antibiotics. This tabular presentation encompasses a range of antimicrobial agents, delineating their Minimum Inhibitory Concentrations (MIC), susceptibility outcomes, sample sizes, and the proportion of resistance detected. Noteworthy is the observation that cefoxitin, benzylpenicillin, and oxacillin all manifested complete resistance, signifying the utter inefficacy of these antibiotics against the tested *S. aureus* strains. Conversely, antibiotics such as gentamicin, tobramycin, levofloxacin, moxifloxacin, tigecycline, fosfomycin,

nitrofurantoin, trimethoprim/sulfamethoxazole, fusidic acid, mupirocin, and ampicillin exhibited total susceptibility, implying the sustained effectiveness of these agents against the isolates in question. Erythromycin and clindamycin displayed variances in resistance and susceptibility. Meanwhile, linezolid, teicoplanin, and rifampicin evidenced some resistance but largely retained their effectiveness. In stark contrast, vancomycin and tetracycline encountered substantial resistance challenges. These findings underscore the critical imperatives of antibiotic stewardship and the prudent selection of antibiotics when addressing *S. aureus* infections. Vigilant monitoring of antibiotic resistance patterns assumes paramount significance in facilitating well-informed clinical decisions and advancing public health endeavors aimed at mitigating this escalating concern.

The table 5 presented in this context provides valuable insights into the occurrence and distribution of antibiotic resistance genes, with a specific focus on resistance to vancomycin, within *S. aureus* isolates sourced from diverse dairy products. This dataset holds significant implications for the comprehension of the potential hazards related to antibiotic resistance in pathogens found in food. Primarily, the tabular data elucidates that a considerable fraction of the 25 *S. aureus* isolates analyzed carries genes conferring resistance to vancomycin. To elaborate, 36% of these isolates possess the Van A resistance gene, and 32% contain the Van B resistance gene. This points to a substantial presence of both resistance determinants within the *S. aureus* population under scrutiny. Secondarily, the distribution of resistance genes across distinct dairy product categories warrants attention. Specifically, cheese, hard cheese, soft cheese, and yogurt display variable levels of vancomycin resistance among the isolates. Notably, soft cheese exhibits the highest proportion of resistant isolates at 32%, while yogurt also manifests a noteworthy share of resistance at 28%. Discerning these disparities in resistance profiles carries implications for enhancing food safety protocols and conducting comprehensive risk assessments. Furthermore, the data signify that while both Van A and Van B resistance genes are present, Van A appears to be marginally more prevalent within this dataset. This observation could steer future research endeavors towards exploring the genetic diversity of resistance genes within *S. aureus* populations and their potential repercussions concerning antibiotic resistance in foodborne pathogens. In conclusion, the insights garnered from this table underscore the critical importance of vigilant surveillance of antibiotic resistance within *S. aureus* isolates extracted from dairy products. The existence of these resistance genes raises legitimate concerns regarding their potential dissemination to human pathogens and the consequent public health implications. These results are similar to the Afshari, *et al*. (2022). They investigated the frequency of Methicillin- and Vancomycin-Resistant *S. aureus* (MRSA, VRSA) and Vancomycin-Resistant Enterococcus (VRE) in hospital food samples in Mashhad, Iran, through studding, 13 hospitals that provided a total of 357 examples of hospital food. The antibiotic resistance patterns of MRSA, VRSA, and VRE strains were analyzed using the disk diffusion methods. The prevalence of *S. aureus* and MRSA were 24.37% (87/357) and 22.98% (20.87), respectively. In addition, the *vanB* gene involved in vancomycin resistance was detected in 1.14% of the *S. aureus* strains. *Enterococci* and VRE had a prevalence of 15.4% (55/357) and 21.81% (12/55), respectively. Meat, chicken barbecues, and salad were the most commonly contaminated samples with *S. aureus*, MRSA, *Enterococci*, and VRE. PCR detected two vancomycin resistance genes, including *van A* (1.81%, 1.55) and *vanC2* (20%, 11.55) genes. MRSA strains revealed the highest resistance against penicillin, erythromycin, clindamycin, azithromycin, tetracycline, and gentamicin. Therefore, further investigative pursuits, encompassing genetic characterization and antibiotic susceptibility assessments, are imperative for conducting a comprehensive evaluation of the significance of these findings and devising strategies to mitigate antibiotic resistance within the food supply chain. Vancomycin is the recommended antibiotic for infections caused by MRSA. Vancomycin-resistant MRSA has been isolated for the first time more than two decades ago(Cong, *et al*. 2020). After this, vancomycin-resistant *S. aureus* (VRSA) has been isolated from more countries of the world such as North America, Europe, Asia, Africa, and South America(Chesneau, *et al*. 2000).

CONCLUSION

Based on the above results, the rate of antimicrobial resistance in *Staphylococcus*

aureus isolates from cheese, soft cheese, had cheese and yogurt was detected. All at once, the presence of Van(A), Van(B) genes determining resistance to vancomycin was confirmed in the isolates. The presence of resistant *S. aureus* shows the risk of the spread of antibiotic resistance in milk and dairy product origin in the local area of Kurdistan region-Iraq.

REFERENCES

Abebe, E., et al. (2020). "Review on major food-borne zoonotic bacterial pathogens." Journal of tropical medicine **2020**.

Abebe, E., et al. (2020). "Review on major food-borne zoonotic bacterial pathogens." **2020**.

Afshari, A., et al. (2022). "Methicillin-and vancomycin-resistant Staphylococcus aureus and vancomycin-resistant enterococci isolated from hospital foods: Prevalence and antimicrobial resistance patterns." **79**(11): 326.

Alakbaree, M., et al. (2022). In-vitro characterization of thrombolytic activity of staphylokinase produced by Staphylococcus aureus S1. AIP Conference Proceedings, AIP Publishing LLC.

Becker, K., et al. (2015). "Staphylococcus, Micrococcus, and other catalase-positive cocci." 354-382.

Boukharouba, A. (2022). Isolation and Identification of Foodborne Pathogens of Special Interest in Food Safety, Universitat Politècnica de València.

Chesneau, O., et al. (2000). "Retrospective screening for heterogeneous vancomycin resistance in diverse Staphylococcus aureus clones disseminated in French hospitals." **45**(6): 887-890.

Cong, Y., et al. (2020). "Vancomycin resistant Staphylococcus aureus infections: A review of case updating and clinical features." **21**: 169-176.

Davies, J. and D. Davies (2010). "Origins and evolution of antibiotic resistance." Microbiology and molecular biology reviews **74**(3): 417-433.

Dizaj, S. M., et al. (2015). "Antimicrobial activity of carbon-based nanoparticles." Advanced pharmaceutical bulletin **5**(1): 19.

Dugassa, J. and N. Shukuri (2017). "Review on antibiotic resistance and its mechanism of development." Journal of Health, Medicine and Nursing **1**(3): 1-17.

Dugassa, J., et al. (2017). "Review on antibiotic resistance and its mechanism of development." **1**(3): 1-17.

Elbehiry, A., et al. (2016). "Performance of MALDI biotyper compared with Vitek™ 2 compact system for fast identification and discrimination of Staphylococcus species isolated from bovine mastitis." **5**(6): 1061-1070.

Hernández-Cortez, C., et al. (2017). "Food poisoning caused by bacteria (food toxins)." Poisoning: From specific toxic agents to novel rapid and simplified techniques for analysis **33**.

Ishaq, A., et al. (2021). "Prospect of microbial food borne diseases in Pakistan: a review." **81**: 940-953.

Lu, Y., et al. (2023). "Modulation of MRSA virulence gene expression by the wall teichoic acid enzyme TarO." **14**(1): 1594.

Manukumar, H. and S. J. S. r. Umesha (2017). "MALDI-TOF-MS based identification and molecular characterization of food associated methicillin-resistant Staphylococcus aureus." **7**(1): 11414.

Mushore, J. and M. Matuvhunye (2013). "Antibacterial properties of Mangifera indica on Staphylococcus

Oliveira, A. P. D. d., et al. (2020). "Characterisation of Staphylococcus aureus strains from milk and goat cheese and evaluation of their inhibition by gallic acid, nisin and velame of the Brazilian caatinga." **73**(2): 345-356.

Patel, R., et al. (2018). "Isolation of Staphylococcus aureus from raw cattle milk and their drug resistance pattern." **7**(2): 836-840.

Thomas, S., et al. (2016). "Future challenges for vaccinologists." 41-55.

Uyttendaele, M., et al. (2015). "Microbial hazards in irrigation water: standards, norms, and testing to manage use of water in fresh produce primary production." **14**(4): 336-356.

Yuan, Q., et al. (2020). "Subgingival Microbes." 145-210.