Methicillin- and Vancomycin-Resistant *Staphylococcus aureus* and Vancomycin-Resistant *Enterococci* Isolated from Hospital Foods: Prevalence and Antimicrobial Resistance Patterns

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Abstract

This study aimed to investigate the prevalence of Methicillin- and Vancomycin-Resistant *Staphylococcus aureus* (MRSA, VRSA) and Vancomycin-Resistant *Enterococcus* (VRE) of hospital food samples in Mashhad, Iran. A total of 357 hospital food samples were collected from 13 hospitals. *Enterococcus* spp. and *Staphylococcus aureus* were identified using conventional cultural techniques following genotypic confirmation by PCR. 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 *vanA* (1.81%, 1.55) and *vanC2* (20%, 11.55) genes. MRSA strains revealed the highest resistance against penicillin, erythromycin, clindamycin, azithromycin, tetracycline, and gentamicin. The VRSA isolates were resistant to penicillin, ampicillin, oxacillin, cefoxitin, clindamycin, erythromycin, gentamicin, and trimethoprim–sulfamethoxazole. Furthermore, VRE isolates exhibited the highest resistance against quinupristin-dalfopristin, erythromycin, and tetracycline. The results of this study indicated that hospital foods might act as a reservoir of *Enterococci* spp. and *S. aureus* strains, which can transfer antibiotic resistance. Moreover, multidrug resistance (MDR) in some MRSA, VRSA, and VRE isolates represents a serious threat to susceptible persons in hospitals.

Introduction

Preparing and providing healthy, nutritious meals for patients is essential in healthcare settings [1–3]. The majority of hospitalized patients suffer from a suppressed immune system are more vulnerable to infection and complications. Thus, hospital foods should have excellent microbial quality [2–4]. Bacteria are significantly responsible for food-borne diseases in hospitals among all pathogenic agents [3]. In this regard, *Staphylococcus aureus* (*S. aureus*) is considered the leading prevalent cause of food-borne outbreaks in

hospitals [3, 4]. *S. aureus* is a gram-positive coccus commonly colonized on the skin and in the respiratory system of healthy humans [3–5]. Moreover, *Enterococci* is another Gram-positive bacterium, which causes food-borne diseases in hospital settings [6], and its species can typically be found in the human intestines [5]. The above-mentioned two microorganisms are potentially opportunistic and problematic pathogens, which have been emerged as the prime causes of healthcare-associated infections, particularly bloodstream infections and associated with the emergence of drug-resistant strains [5]. Their antibiotic-resistant strains have higher pathogenicity and cause more severe diseases than their antibiotic-susceptible strains [3, 4]. The methicillin and vancomycin resistance strains of these two bacteria have attracted worldwide concern as major phenotypes of resistant bacteria [7]. *S. aureus* isolates recovered from food samples and clinical infections harbored a high prevalence of resistance against routinely applied antibiotics, especially methicillin [3, 4]. Furthermore, documented data have shown that around 50–70% of *S. aureus* strains obtained

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from the hospital environment were methicillin resistant [4]. Methicillin-resistant *S. aureus* (MRSA) strains are typically resistant to aminoglycosides, macrolides, lincosamides, and all classical β-lactam antimicrobial drugs [8]. In addition, recent reports about vancomycin-resistant *Enterococci* (VRE) have represented the high incidence of resistance to commonly used groups of antimicrobial agents, especially glycopeptides, β-lactams, lincosamides, streptogramins, and aminoglycosides [9]. Meanwhile, vancomycin-resistant *S. aureus* (VRSA) has emerged as a food safety and medical concern [10]. The morbidity and mortality rates of antibiotic-resistant organisms have recently increased among hospitalized patients [11].

Accordingly, consuming contaminated food with multidrug-resistant pathogens poses a high risk for patients, who are more vulnerable to infection and its complications [12]. Given the lack of food epidemiological surveys on the prevalence of these microorganisms in hospital foods, this study aimed to assess the prevalence rate and antibiogram-resistant patterns of MRSA, VRSA, and VRE isolates recovered from various types of cooked and ready-to-eat food samples from hospitals in Mashhad, Iran.

Materials and Methods

Sample Collection

A total of 357 hospital food samples (321 cooked foods and 36 ready-to-eat salads) were aseptically collected from 13 hospitals in Mashhad city, Iran, from August 2019 to January 2020. The collected samples were immediately transferred to the laboratory of the Nutrition Department, Faculty of Medicine, University of Medical Sciences, Mashhad, Iran, in a cooler with ice packs and examined within 1–2 h. The samples included meat barbecue ($n = 36$), chicken barbecue ($n = 36$), cooked meat ($n = 36$), cooked chicken ($n = 36$), fried fish ($n = 33$), cooked rice ($n = 36$), dill rice ($n = 36$), mixed or bean rice ($n = 72$, mixed rice cooked with either red meat, poultry, vegetables, and/or beans), soup ($n = 36$), and salad ($n = 36$).

Enumeration, Isolation, and Identification of *S. aureus* and *Enterococci*

About 10 g of each sample was placed into sterile plastic bags. Then, 90 mL of sterile buffered peptone water 0.1% (w/v) (BPW, Merck, Germany) was added to the samples, and the mixture was thoroughly homogenized. Then, tenfold serial dilutions of the homogenates were prepared, and the spread plate technique was employed to enumerate *S. aureus* and *Enterococci*. A total of 0.1 mL of each dilution (10^{-1} to 10^{-4}) was inoculated onto the Baird-Parker

agar (BPA, Merck, Germany) plates, supplemented with 1% potassium tellurite solution and 5% egg yolk emulsion in terms of *S. aureus* enumeration. The inoculums were evenly smeared using a sterile L-shaped glass rod. The inoculated plates were then kept in an incubator at 37 °C for 24 h. The obtained colonies were enumerated, and *S. aureus* was verified by a DNase test agar and a tube coagulase test. Then, 0.1 mL of each dilution (10^{-1} to 10^{-4}) was inoculated onto KF-*Streptococcus* agar (KF, Conda, Spain) media, supplemented with 0.1 g/L 2,3,5-triphenyl tetrazolium chloride (TTC, Merck, Germany), and then incubated at 37 °C for 24–48 h in terms of *Enterococci* spp. Enumeration. Typical colonies of *Enterococci* spp. were carefully counted on KF plates after incubation, and *Enterococci* colonies were verified using catalase and esculin hydrolysis tests.

At this stage, 10 g of each sample was homogenized for isolation of *S. aureus*. Then, 90 mL of Trypticase Soy Broth (TSB, Merck, Germany) and 10% w/v sodium chloride (NaCl, Merck, Germany) were added to the homogenized samples, and the mixture was incubated for 18–24 h at 37° C. Following enrichment, a loopful of these broth cultures were streaked onto selective agar media (BP agar containing egg yolk tellurite emulsion (50 mL/L)) and incubated at 37 °C for 24 h.

About 1 g of homogenized food samples was added to test tubes containing 9 mL of Azide Dextrose broth and 6.5% w/v sodium chloride (NaCl, Merck, Germany) for isolation of *Enterococci* spp. Then, the mixture was mixed in a vortex mixer for one minute. In the next stage, the tubes were incubated for 24 h at 37 °C. One loopful of each enriched sample in broth was streaked onto KF agar containing triphenyl tetrazolium chloride (0.1 g/L) after incubation, and the plates were then incubated aerobically at 37 °C for 24–48 h.

Typical morphology of this bacteria (i.e., small, circular, convex, gray to black colonies, and with or without opaque halo) was used to identify *S. aureus,* colonies based on gram staining, catalase reaction (using 0.3% hydrogen peroxide), coagulase test (rabbit plasma), oxidase test, mannitol fermentation on Mannitol salt agar (Conda, Spain), deoxyribonuclease (DNase, Conda, Spain) test, and carbohydrate (xylose, sucrose, maltose, trehalose, and lactose) fermentation tests.

Gram staining, catalase activity, esculin hydrolysis on bile esculin azide agar (Quelab, Canada), and carbohydrate (arabinose, lactose, sorbose, mannitol, and sorbitol) fermentation tests were conducted on suspected colonies to identify *Enterococci* spp. These suspected colonies are observed in red, pink, or dark red and with variations in diameter from 0.3 to 2 mm. All confirmed pure colonies were kept frozen at − 20 °C in Brain Heart Infusion (BHI, Merck, Germany) broth containing 15% (v/v) glycerol (Merck, Germany) until molecular and antibiotic susceptibility testing. *S. aureus*

ATCC 25923 and *Enterococcus faecalis* ATCC 29,212 strains were used as control organisms.

Phenotypic Identification of MRSA, VRSA, and VRE Isolates

MRSA strains were detected by cefoxitin (30 μg) and oxacillin (1 μg) susceptibility tests after isolation. Isolates with inhibition zone size ≤ 21 mm and cefoxitin ≤ 10 mm with oxacillin were considered resistant. Moreover, isolates with inhibition zone size ≤ 9 mm around a 30 μg disk of vancomycin were classified as VRSA. VRE strains were detected by testing with vancomycin disk with an inhibition zone ≤ 14 mm. All tests were carried out on these isolates using the Kirby–Bauer disk diffusion technique and interpreted based on the Clinical Laboratories Standards Institutes guidelines (CLSI).

DNA Extraction

A genomic DNA isolation kit (DENAzist, Iran) was used to extract chromosomal DNA from the bacterial cells. Then, the quality (A260/A280) and concentration of extracted DNA were quantified using a NanoDrop 2000 spectrophotometer (NanoDrop, Thermo Scientific, USA).

Detection of Antibiotic Resistance Genes by PCR Assay

All phenotypically identified bacterial isolates were genotyped using the PCR-based detection of their specified genes (MRSA, VRSA, and VRE strains) with specific oligonucleotide primers. Supplementary Table 1 displays the PCR primers and products used in this study (Gen Bank Accession Number NC_003923M, 293 bp). The primer sets and PCR cycling protocols used in the PCRs were selected from earlier reports (Supplementary Table 1). All PCR reactions were applied in a thermocycler (T100, Bio-Rad, USA). The PCR Thermal program used in this study is represented in Supplementary Table 2. Oligonucleotides were manufactured by Pishgam Company (Tehran, Iran), and the amplified products were visualized under UV light in a gel documentation imaging system (Quantum ST4, Vilber Louma, Germany). In this molecular assay, we used the positive controls (ATCC 25923 and ATCC 29212) and positive clinical cultures (MRSA- or VRE-positive clinical cultures) of specimens obtained from hospitalized patients at Imam Reza hospital, Mashhad, Iran for positive controls. Moreover, each run included a negative control, containing reaction mixtures lacking DNA templates.

Antimicrobial Susceptibility Pattern of MRSA, VRSA, and VRE Isolates

Antimicrobial susceptibility patterns of all isolates, identified as resistant, were studied by the simple disk diffusion technique (Kirby–Bauer) on Mueller–Hinton agar (MHA, Quelab, Canada) using disk containing antibiotics (Cyprus, Belgium, and Padtan Teb, Iran), recommended by CLSI. Suspensions equivalent to 0.5 McFarlands were inoculated on the surface of MHA plates and then incubated for up to 24 h at 37 °C. Following this period, the growth inhibition (halo) diameters around each disk were measured and interpreted according to the CLSI criteria. *Enterococci faecalis* ATCC 29212 served as a quality control strain for all susceptibility tests. All MRSA isolates were examined for susceptibility to penicillin (10 U), gentamicin (10 μg/disk), amikacin (30 μg/disk), kanamycin (30 μg/disk), azithromycin (15 μg/disk), erythromycin (15 μg/disk), tetracycline (30 μg/disk), doxycycline (30 μg/disk), ciprofloxacin (5 μg/disk), levofloxacin (5 μg/disk), clindamycin (2 μg/disk), trimethoprim–sulfamethoxazole (25 μg/disk), chloramphenicol (30 μg/disk), rifampin (5 μg/disk), and vancomycin (30 μg).

In addition, cefoxitin (30 μg/disk), oxacillin (1 μg/disk), penicillin (10 U), ampicillin (10 μg/disk), gentamicin (10 μg/disk), erythromycin (15 μg/disk), clindamycin (2 μg/disk), trimethoprim–sulfamethoxazole (25 μg/disk), chloramphenicol (30 μg/disk), quinupristin–dalfopristin (15 μg/disk), and teicoplanin (30 μg/disk) were tested for VRSA isolates.

Moreover, the susceptibility of VRE isolates to penicillin (10 U), ampicillin (10 μg/disk), erythromycin (15 μg/disk), tetracycline (30 μg/disk), doxycycline (30 μg/disk), ciprofloxacin (5 μg/disk), levofloxacin (5 μg/disk), rifampin (5 μg/disk), quinupristin-dalfopristin (15 μg/disk), and teicoplanin (30 μg/disk) was assessed.

Statistical Analysis

The data were analyzed based on descriptive statistical analysis with Excel software (version 2019) and SPSS software (Version 18.0; IBM, Armonk, USA). The data are expressed as means $\pm$ standard deviation (*SD*) with two repetitions.

Results

Enumeration of *S. aureus* and *Enterococci*

Table 1 shows the frequency of food samples contaminated above Iranian standards (> 102). As shown in Table 1, 8.12% (29/357) and 9.52% (34/357) of food samples had an unacceptable level of *S. aureus* and *Enterococci,* respectively, compared to the Iranian standard level. Moreover, the salad had the highest frequency of over-contamination for *S.*

Table 1 Frequency of food samples with contamination above the Iranian standard level ($> 10^2$)

Food sample	*S. aureus* Number of samples (percent)		*Enterococci* Number of samples (percent)		
	(Between 10^2 and 10^3 CFU/g)	(Between 10^3 and 10^4 CFU/g)	(Between 10^2 and 10^3 CFU/g)	(Between 10^3 and 10^4 CFU/g)	(Between 10^4 and 10^5 CFU/g)
Meat barbecue	2 (5.55)	1 (2.77)	2 (5.55)	–	1 (2.77)
Chicken barbecue	2 (5.55)	–	3 (8.33)	–	1 (2.77)
Cooked meat	1 (2.77)	–	1 (2.77)	–	–
Cooked chicken	4 (11.11)	–	–	–	–
Fried fish	2 (6.06)	–	–	–	–
Cooked rice	3 (8.33)	–	–	–	–
Dill rice	3 (8.33)	–	–	–	–
Bean rice	–	–	–	–	–
Soup	4 (11.11)	–	2 (5.55)	–	1 (2.77)
Salad	7 (19.44)	–	16 (44.44)	5 (13.88)	2 (5.55)
Total	29 (8.12)		34 (9.52)		

aureus with 19.44% (7/36) and *Enterococci* with 63.88% (23/36).

Prevalence of *S. aureus*, MRSA, and VRSA Strains

Based on our findings, *S. aureus* was isolated from 24.37% of the total analyzed samples (87 out of 357). The percentage of *S. aureus*, MRSA, and VRSA is shown in Supplementary Fig. 1 for various hospital food samples. In this figure, the meat barbecue showed the highest frequency of *S. aureus* (33.33%, 12/36), followed by the chicken barbecue (30.55%, 11/36) and salad (30.55%, 11.36). In contrast, dill rice and cooked meat samples presented the lowest frequency (16.66%) after cooked rice (19.44%).

The antibiotic susceptibility test (phenotypic assay) showed 20 isolates (22.98%) resistant to methicillin classified as MSRA from 87 *S. aureus* isolates recovered from various types of hospital food samples. The PCR assay confirmed these 20 isolates as MRSA by detecting the *mecA* gene. As shown in Supplementary Fig. 1, the frequencies of MRSA-positive samples were 2.77 (1/36), 2.77 (1/36), 2.77 (1/36), 2.77 (1/36), 5.55 (2/36), 6.06 (2/33), 8.33 (3/36), 11.11 (4/36), and 13.88% (5/36) for dill rice, cooked rice, cooked meat, cooked chicken, soup, fried fish, salad, meat barbecue, and chicken barbecue, respectively. The highest percentage of MRSA contamination was found in chicken and meat barbecues. In comparison, cooked chicken samples had the lowest prevalence of MRSA. Furthermore, the *mecA* gene was not detected in mixed rice (bean rice) samples.

According to the results of the vancomycin susceptibility test, all 87 isolates of *S. aureus* were susceptible to vancomycin. However, PCR results found that one isolate of *S. aureus* (1.14%) carried the *vanB* gene, obtained from meat barbecue and classified as VRSA, regardless of the

vancomycin disk diffusion test results. Generally, 98.85% of the samples were negative for VRSA test.

Prevalence of *Enterococci* and VRE Strains

According to the results, *Enterococci* spp. were recovered from 15.40% of the total collected samples (55/357). The percentage of *Enterococci and* VRE is represented in Supplementary Fig. 2 for various hospital food samples. As shown in this figure, RTE salad exhibited the highest prevalence of *Enterococci* spp. (80.55%, 29/36) after chicken barbecue (30.55%, 11/36). In contrast, the lowest frequency was fried fish samples (3.03%, 1/36), and the cooked rice and cooked chicken samples were all negative.

According to the vancomycin disk diffusion method, 7.27% (4/55) of *Enterococci* isolates were VRE, including 1.81% of *Enterococci* isolates (1/55) exhibited complete resistance to vancomycin (zone diameter ≤ 14 mm). In comparison, 5.45% of *Enterococci* isolates (3/55) showed an intermediate resistance pattern to vancomycin (zone diameter 15–16 mm). Complete resistance to vancomycin was detected in 2.77% (1 of 36) of isolates obtained from chicken barbecue samples. In addition, the intermediate resistance to vancomycin was observed in 2.77% (1 of 36) and 5.55% (2 of 36) of isolates obtained from soup and RTE salad samples. The results of PCR assays indicated 21.81% (12/55) positive isolates for *van* genes (*vanA* and *vanC2/3*), which are classified as VRE. V*anC2/3* (91.66%, 11/12) was the most frequently detected resistance gene among detected resistance genes. The *vanA* gene had the lowest prevalence (8.33%, 1/12) among detected resistance genes. In addition, there were no positive results for *vanB* and *vanC1* genes, and the same isolate, which showed complete resistance to vancomycin, was positive for the *vanA* gene. Moreover, all

3 VRE isolates, which exhibited intermediate resistance to vancomycin, were positive for the vanC2/3 gene. According to Supplementary Fig. 2, RTE salad samples presented the highest prevalence of VRE-positive samples (22.22%, 8/36) after chicken barbecue (5.55%, 2/36), meat barbecue (2.77%, 1/36), and soup (2.77%, 1/36).

Antibiotic Resistance Patterns of MRSA

Supplementary Fig. 3 displays the antibiotic resistance patterns of MRSA isolated from food samples. The results indicated that MRSA strains harbored the highest prevalence of resistance toward antibiotic agents, including penicillin (100%), erythromycin (90%), clindamycin (90%), azithromycin (90%), tetracycline (75%), gentamicin (75%), doxycycline (65%), and trimethoprim–sulfamethoxazole (50%). On the contrary, MRSA strains harbored the lowest prevalence of resistance toward antibiotics, such as levofloxacin (5%), kanamycin (10%), chloramphenicol (10%), ciprofloxacin (40%), and amikacin (40%) antibiotic. On the other hand, all of the MRSA isolates were sensitive to rifampin (100%) (Supplementary Fig. 3).

Antibiotic Resistance Patterns of VRSA

Supplementary Fig. 4 illustrates the patterns of antibiotic resistance of VRSA isolated from food samples. Antibiogram results revealed that the isolated VRSAs were 100% resistant to ampicillin, oxacillin, cefoxitin, erythromycin, clindamycin, gentamicin, and trimethoprim– sulfamethoxazole and 100% sensitive to teicoplanin and quinupristin-dalfopristin (Supplementary Fig. 4).

Antibiotic Resistance Patterns of VRE

Supplementary Fig. 5 shows the patterns of antibiotic resistance of VRE isolated from food samples. According to these findings, VRE strains showed a high prevalence of resistance to quinupristin–dalfopristin (100%), erythromycin (83.33%), tetracycline (83.33%), doxycycline (66.66%), and rifampin (66.66%) antibiotic agents. Meanwhile, a low prevalence of resistance was observed against ampicillin (8.33%), teicoplanin (8.33%), levofloxacin (8.33%), penicillin (25%), and ciprofloxacin (33.33%) antibiotic agents.

Discussion

Antibiotic resistance is now a global concern due to the indiscriminate use of antibiotics in agriculture, the environment, veterinary medicine, and medicine. Antibiotic-resistant isolates may be able to transmit resistance genes to bacteria in the human body [13]. Therefore, hospital meals should have an acceptable level of health quality due to the weak immune system of hospitalized patients [2, 3, 14]. Thus, this study dictated the prevalence and antibiotic susceptibility pattern of *S. aureus* and its resistant isolates (MRSA and VRSA), as well as *Enterococci spp.,* and its resistant isolate (namely VRE) among hospital food samples.

The results of enumeration of *S. aureus* in hospital food samples showed that 8.12% (29/357) of the food samples had contamination above the allowable level according to Iranian national standards. The contamination between 10^2 to 10^3 and 10^3 to 10^4 CFU/g was observed in 6.54% (21/321) and 0.31% (1/321) of all cooked food samples. In addition, 19.44% (7/36) of salad samples had contamination between 10^2 and 10^3 CFU/g. The microbial count results of a study [15] in turkey showed that 2.4% (13/530) out of 530 hospital food samples were infected with *S. aureus,* above the Turkish national standard. Moreover, 2.1% of cooked food samples had contamination between 10^2 and 10^3 CFU/g, and 11.4% of salad samples were contaminated with 10^3 to 10^4 CFU/g. No contamination of this bacterium was observed in soup and cooked rice samples. However, cooked rice and soup samples were positive in terms of count.

Our results showed that *S. aureus* prevalence in different hospital foods was 24.37% (87/357). In a study performed in Isfahan in 2018, 47 out of 457 hospital food samples (10.28%) were contaminated with *S. aureus,* higher than our results [3]. Another survey carried out in Tehran in 2013 showed that two samples out of 44 hospital food samples (4.54%) were contaminated with *S. aureus*, which was lower than our results [16]. The prevalence of this bacterium was reported from 20 to 25% in Iran in food samples of places, such as restaurants and fast foods [17, 18]. These differences could be due to sample size and type, as well as hygienic conditions in the hospital kitchen, personal staff hygiene, diagnostic methods, and geographical area [19]. There are several reasons for the high prevalence of *S. aureus* in hospital food samples, such as nurses and hospital staff given that *S. aureus* is one of the most common microorganisms, which is colonized in the nasal cavity and on the body surface, approximately 50% of the human population carries *S. aureus* [20]. Moreover, other factors, including low quality and inaccurate washing of raw materials, insufficient time and temperature for cooking and storing food (especially meat), use of contaminated equipment for cooking, and lack of personal hygiene, reduce hospital food quality [2, 3].

In the present study, the meat barbecue sample had the highest contamination rate (13.79%, 12.87), followed by a chicken barbecue and salad samples (12.64% for each, 11.87). In a study conducted in Esfahan in 2017 showed that the highest infection of *S. aureus* was observed in meat barbecue, followed by a chicken barbecue and salad samples, which was consistent with our result [4]. Similarly, Ranjbar et al. (2017b) found that the most contamination

rate was reported in chicken and meat barbecue samples [17]. According to the study, beef and poultry meat are the primary sources of *S. aureus* pathogens in these samples [3, 21]. In addition, low cooking temperature and time for meat would be the second reason [17].

The findings of the present study showed that the lowest level of contamination was in the samples of vegetable dill rice (6.89%, 6/87), cooked meat (6.89%, 6/87), and cooked rice (8.04%, 7/87), which could be due to their preparation conditions at higher temperatures and times to kill the pathogenic bacteria [2, 3].

Moreover, 22.98% (20/87) of *S. aureus* isolates were classified as MRSA, harboring the *mecA* gene. In addition, the MRSA prevalence in the tested food samples was 5.60% (20.357). In a study performed in Tehran in 2013, 1 sample out of 44 hospital food samples (2.27%) was MRSA positive [16]. Another author in 2017 in the United States demonstrated that 29 out of 910 hospital food samples (3.2%) were MRSA positive [22]. Unlike the present study, the prevalence of MRSA strains in these two mentioned surveys was lower than our results. According to recent study about the prevalence of MRSA in food products in other settings, five out of the 120 utensil samples collected from hotels and non-hotel establishments were positive for the *mecA* gene. At the same time, no positive *mecC* gene were found [23]. In another study in Isfahan, Iran, 83 out of 119 (14.31%) restaurant food samples were positive for the *mecA* gene. The prevalence of MRSA strains in this study was higher than our study [17]. In addition, a higher prevalence of MRSA has been reported in various studies in other parts of Iran as much as 22.65% [18] and 37.4% [24]. The differences between their results and ours could be explained by the fact that in their studies, MRSA strains were isolated from processed (packaged hamburgers) [18] as well as raw (beef, chicken, and turkey) [24] food samples. In the present study, chicken barbecue samples (25%, 5/20), meat barbecue (20%, 4/20), and salad (15%, 3/20) samples had the highest presence of MRSA strains, respectively. Contrary to our results, a study conducted on hospital foods in Brazil and reported the highest rates of MRSA contamination in fish foods [21]. Moreover, an investigation which performed in 2017 reported that all isolates of *S. aureus* obtained from hospital food samples were resistant to methicillin, and the highest prevalence of MRSA strains was in meat barbecue samples (16.12%, 5/31), chicken barbecue (8.53%, 7/82), and salad (7.14%, 4/56), which were lower than our findings [4]. The high prevalence of MRSA in chicken barbecue samples may be due to the high use of human-based antibiotics (especially methicillin) in Iranian poultry husbandry as a therapeutic solution, as well as growth stimulants, which kill other bacterial competitors. Thus, *S. aureus* can grow and multiply more efficiently [17].

The results of the disk diffusion method showed that all MRSA isolates were resistant to penicillin antibiotics, which was consistent with Dehkordi et al. (2017) [4], Rahimi et al. (2019) [24], and Jackson et al. (2013) [25]. The high frequency of penicillin resistance in food samples may indicate the indiscriminate use of ampicillin in treating livestock and poultry infections [24]. 90% of MRSA isolates were resistant to erythromycin, which is similar to the results of Dehkordi et al. (2017) [4] and Rahimi et al. (2019) [24]. The high frequency of erythromycin resistance may reflect the widespread use of erythromycin and spiramycin antibiotics as a growth promoter in livestock and poultry feed in Iran [24]. In our findings, the resistance to clindamycin was higher than other studies (90%). In Rahimi et al. (2019) [24], 73% of MRSA isolates were resistant to clindamycin. MRSA strains isolated from the food samples of our study had high resistance to human antibiotics, such as trimethoprim–sulfamethoxazole, ciprofloxacin, and azithromycin, which can indirectly confirm the human origin of these resistant bacteria [17]. Conversely, another study indicated that all *S. aureus* isolates from 120 hotel and non-hotel samples (i.e., serving utensils) were sensitive to ciprofloxacin [23]. The resistance to trimethoprim–sulfamethoxazole in our study was 50%, which was consistent to studies which were conducted in Iran [3, 24]. MRSA strains were resistant to azithromycin, which was in line with Ranjbar et al. (2017b) [17], and Dehkordi et al. (2017) [4] reported that 71.8% and 59.45% of isolates were resistant to this antibiotic. Azithromycin and ciprofloxacin are human antibiotics and are not common in veterinary medicine. The high prevalence of resistance to these antibiotics may indicate cross-contamination through infected individuals and staff [3].

According to the present study, 75% of MRSA isolates were resistant to tetracycline. These results are consistent with those of Ranjbar et al. (2017b) [17] and Rahimi et al. (2019) [24] in Iran, who found that MRSA strains isolated from food samples were 92.77% and 65% resistant to tetracycline, respectively. A significant amount of antibiotics, such as tetracyclines and quinolones, are found in the soil due to the use of organic fertilizers in farms and irrigation with wastewater or treated water, which can increase the presence of these antibiotics in the food chain [12]. In our study, the resistance of MRSA strains to chloramphenicol was 10%, which was consistent with previous study conducted in China [26]. Additionally, all *S. aureus* isolates (100%) from 120 hotel and non-hotel samples (e.g., serving utensils) were chloramphenicol resistant [23]. The use of chloramphenicol in veterinary medicine is prohibited, and the high prevalence of resistance to chloramphenicol has been attributed to its illegal use for treatment, especially in poultry farms [17].

None of the S. aureus isolates were categorized as VRSA based on the disk diffusion method, and none of the S.

aureus isolates were categorized as VRSA. The PCR results elucidated that one isolate of *S. aureus* (1.15%, 1.87) was classified as VRSA due to having the *vanB* gene. Furthermore, this isolate was also resistant to methicillin (MRSA). Another investigator in Shiraz in 2014 [27] showed that six strains of *S. aureus,* which had the *vanA* gene, did not show resistance in phenotypic tests (vancomycin disk diffusion test, vancomycin agar screen, and minimum inhibitory concentration of vancomycin). However, another study identified 2 of 85 isolates of *S. aureus* isolated from minced meat as MRSA strains using the vancomycin-resistant disk diffusion method. However, PCR results found no *vanA* and *vanB* genes in these isolates [19], indicating that phenotypic methods, such as disk diffusion, and screen agar may be subject to errors and in vitro mutations. Therefore, the use of more standard phenotypic methods, E-test antibiotic strips, and molecular methods, such as PCR for resistance gene tracing, is necessary to confirm 100% of VRSA strains [28]. Moreover, a recent author revealed that one isolate of *S. aureus* (7.69%) contained VRSA genes, such as *vanA*, *vanB*, and *vanC2/3*, but not vanC1. The *vanB* gene was observed in VRSA isolates, which was in accordance with our results. Moreover, isolates, identified as MRSA, were also VRSA [29]. Additionally, another author has shown that four of the five *vanC*-positive isolates were positive for the *mecA* gene, and MRSA and VRSA are a threat to public health in food processing environments [23].

In the present study, VRSA isolates were resistant to the antibiotics, like penicillin, ampicillin, clindamycin, erythromycin, trimethoprim, sulfamethoxazole, gentamicin, cefoxitin, and oxacillin. Additionally, they were sensitive to teicoplanin, quinupristin–dalfopristin, and vancomycin. Similar to our study, in a study carried out in Brazil [29] reported VRSA isolates resistant to the antibiotics, like penicillin, oxacillin, and clindamycin. However, unlike our study, all isolates were sensitive to gentamicin and resistant to teicoplanin. Saadat et al. [27] showed that VRSA isolates were sensitive to the antibiotic quinupristin–dalfopristin and resistant to the ampicillin antibiotic, which was consistent with our study.

According to Iranian national standards, the *Enterococci* count showed that 9.52% (34/357) of the hospital food samples had contamination above the permissible level. Moreover, 63.88% (23/36) of salad samples had more than 10^2 bacteria per gram, which was in line with the result of another study [30].

The presence of *Enterococci* in food is a controversial and worrying issue because this bacterium is an indicator of fecal contamination in food [31]. This study showed that 15.40% (55/357) of all hospital food samples were infected with Enterococci. Unlike the present study, in another survey performed in Tehran in 2013 none of the 44 food samples were infected with *Enterococci* [16]. In our study, salad samples (55.72%, 29/55) had the highest contamination rate with *Enterococci*. In addition, the prevalence of *Enterococci* in salad samples in the present study was 80.55% (29/36). In a recent study in Tehran in 2012 [32], salads had the highest prevalence of *Enterococci* infection (29.17%, 7/24), which was lower than our results. Vegetables and salads can be a source of contamination of intestinal bacteria, such as *Enterococci* because of lack of proper washing and raw consumption [30, 33]. Secondary food contamination is another most important cause of infection due to improper food preparation [34]. On the other hand, *Enterococci* can grow under a wide range of environmental conditions (temperature, salt, and pH) considering survival factors in cooked foods [33, 35].

In the present study, PCRs showed that 21.81% (12/55) of *Enterococci* species isolated from hospital food samples had *van* genes, which were identified as VRE. In addition, the prevalence of VRE in all hospital food samples was 3.36% (12/357). Unlike our study, in an investigation which was performed in the United States in 2017, the prevalence of VRE isolates was 2.4% (22/910) in hospital food samples, which was lower than ours [22]. A higher prevalence of VRE was reported by Novise et al. (2005), who found that 57.31% of *Enterococci* isolated from poultry meat (47/82) were vancomycin resistant [36].

The emergence of antibiotic-resistant *Enterococci* in food samples is due to the widespread use of antibiotics in agriculture and veterinary medicine [37]. Crops may also be contaminated by environmental sources, such as human and animal feces [38]. *Enterococci* can adapt to the environment, which are resistant to widely used antibiotics, such as tetracycline, lincomycin, erythromycin, and compounds used as growth stimulants (AGPs) in veterinary medicine [39, 40]. In the present study, all VRE isolates were resistant to quinupristin–dalfopristin. According to Raafat et al. (2016) [9], 100% of VRE isolates were resistant to quinupristin–dalfopristin, which was similar to our result, as well as some studied in Portugal, Egypt, and Italy [9, 36, 41]. Quinupristin–dalfopristin resistance may be due to the use of virginiamycin in normal animal weight gaining. Virginiamycin is a streptogramin antibiotic (quinupristin–dalfopristin) used as a growth stimulant in many countries, including Iran [9]. This antibiotic causes cross-resistance to quinupristin–dalfopristin, which has been banned in the EU since 1999 [9]. Our results also indicated the high frequency of resistance to erythromycin (83.33%) in most VRE isolates, which was consistent with the results of other researchers, including Jung et al. (2007) [42] and Fracalanzza et al. (2007) [34]. The high frequency of erythromycin resistance in food isolates may be due to the presence and proliferation of erythromycin-resistant plasmids and transposons among enterococcal species [43]. Furthermore, resistance to tetracycline, doxycycline, and ciprofloxacin in our study

was 83.33%, 66.66%, and 33%, which could be due to the widespread use of tetracyclines and quinolones in animal husbandry [36]. Moreover, 66.66% of VRE isolates were resistant to rifampin, similar to Kim et al. (2017) [38] and Jahansepas et al. (2020) [37]. In addition, the sensitivity of VRE isolates to penicillin was reported in many studies [40] so that 75% of the isolates were sensitive to this antibiotic in our findings. The differences in antibiotic resistance patterns are due to factors, such as genetic changes in isolates, rate of antibiotic use, and availability of each antibiotic. This finding could indicate the most widely used antibiotic in an area [44].

Conclusion

The results showed a significant prevalence of *S. aureus* and *Enterococci* and their antibiotic-resistant strains in hospital food samples. Therefore, careful studies are required in education, observance of hygienic principles during food processing and storage, taking more severe control measures, and adherence to good veterinary practices regarding antibiotics administration. Complete cooking of food and prevention of cross-contamination seems to be the most effective way to prevent the occurrence of antibiotic-resistant strains in hospital food.

Limitation of the Study

This study had some limitations, including the lack of using MIC tests to identify resistant strains of *S. aureus* and *Enterococcus*. Furthermore, molecular identification of bacterial isolates was not performed, although the phenotypical tests in addition to the positive controls (ATCC 25923 and ATCC 29212) and positive clinical cultures (MRSA- or VRE-positive clinical cultures) of specimens obtained from hospitalized patients at Imam Reza hospital, Mashhad, Iran, were used as positive control in this study.

Declarations

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