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DETECTION OF *VANA* OF VANCOMYCIN RESISTANCE *S. AUREUS* ISOLATED FROM MEAT SAMPLES COLLECTED TEHRAN

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ABSTRACT

Staphylococcus aureus to be one of the leading causes of food-borne diseases. Types of meat and meat products are often contaminated with resistant strains of this bacterium. Foodstuff contamination may occur directly from infected food-producing animals or may result from poor hygiene during production processes, or the retail and storage of foods, since humans may carry the microorganism. The number of *S. aureus* strains that exhibits antimicrobial-resistance properties has increased, together with the potential risk of transmitting the same properties to the human microflora via foods or inducing infections hard to be tread. Of 186 meat samples consisting of 89 (47.85 %) from hen/chicken meat, 30 (16.13 %) from turkey meat, 47 (25.27 %) from calf/cow meat and 20 (10.75 %) from sheep meat examinated for the presence *S. aureus*. All isolates were tested for resistance to vancomycin by disk diffusion test and MIC methods according to CLSI standards. Resistant and Intermediate isolates were tested for Presence the vanA gene. *S. aureus* strains was isolated from 46 (24.73 %) samples consisting of 19 (41.30%) from hen/chicken meat, 10 (21.74 %) from turkey meat, 11 (23.91 %) from calf/cow meat and 6 (13.05 %) from sheep meat. There were a number of isolates VRSA (n=24 , 52.17 %), VISA (n=9 , 19.57%) and VSSA (n=13, 28.26%). Among all strains were a number containing the vanA gene (n=16 , 34.78%). Results show clearly the potential risks behind poor sanitary conditions during meat processing. Considering that Vancomycin resistance strains within human society could be originated from food materials that have been produced and processed under poor conditions.

Keywords: *Staphylococcus aureus*, vancomycin Resistance, *vanA*, Meat samples, PCR.

INTRODUCTION

Antibiotic resistance has been reported from vast variety of food borne disease agent including *Staphylococcus aureus* [1]. *S. aureus* is a versatile pathogen of humans and animals and causes a wide variety of diseases ranging in severity from slight skin infection to more severe diseases such as pneumonia and septicaemia [2]. Several virulence factors implicated in the pathogenesis of *S. aureus* strains, have been described in the literature such as thermonuclease, hyaluronidase, lipases and hemolysins, which are involved in tissue invasion of the host cells. Perhaps the most notable virulence factors associated with this microorganism are the heat-stable enterotoxins (SEs), that cause the sporadic food-poisoning syndrome and the toxic shock syndrome toxin 1 (TSST-1), which diminishes the immune response of a colonized host [3]. On the other hand the rapid evolution of antibiotic resistance in *S. aureus* is of considerable concern [4]. From antibiotics that have been used for the clinical treatment of *S. aureus* infections include fusidic acid, rifampicin and vancomycin [5]. Vancomycin resistance genes have been disturbed within wide spectrum of enteric pathogens [6] [7]. Vancomycin-resistant *S. aureus* (VRSA) was isolated in Japan in 1997, and soon found elsewhere, including the United states. It has been suggested that VRSA has developed by acquiring the vanA operon (gene cluster) from vancomycin-resistant enterococci (VRE) [8]. Transfer of antibiotic-resistant bacteria to humans (or their antibiotic resistance genes to pathogens) via the food chain has already bean reported [9]. Antimicrobial are used extensively in food animal protection where they are often applied subtherapeutically for growth promotion and routine disease prevention[10]. According to reservoir hypothesis, colonic bacteria normally residing in colon including those. That cloud act as opportunistic pathogens and those that are truly non-pathogenic, exchange DNA with one other, and act as reservoirs for resistance genes that can be acquired from ingested bacteria [11]. The aim of this study was to evaluate the risk of exposure to vancomycin-resistant *Staphylococcus aureus* from raw meats in Tehran.

MATERIALS AND METHODS

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Collection of samples

From May to July 2012, 186 of chicken, poultry, cow and sheep meats samples were collected from Tehran.

Bacterial strains

A total of forty six *Staphylococcus aureus* strains by growth in Baird parker agar, Blood agar, and by was used from Microscopic View, Catalase, Coagulase, Manitol salt agar tests isolated from meat samples.

TEST FOR ANTIBIOTIC SUSCEPTIBILITY

Disk diffusion method

S. aureus isolates were tested for their susceptibility to vancomycin by use of disk diffusion technique. Bacterial strains were inoculated into Mueller Hinton Broth (MHB) (with equal concentration 0.5 MacFarland standard). Mueller Hinton Agar (MHA) plates were then inoculated with this bacterial suspension and spread as evenly as possible. One disk vancomycin (van30) was then placed on each of the inoculated MHA plates and inoculated at 37 ^{o}C for 18-24 hours. After incubation, the diameter of the inhibition zone for vancomycin was measured to the nearest millimetre. Isolates were described either as resistant (not completely inhibited, ≤12 mm), intermediately resistant (not completely inhibited, 13-14 mm), or sensitive (appropriately inhibited, ≥15 mm), based on the size of inhibition zones [5], [12] and [13].

Minimal Inhibitory Concentration (MIC) method

The MIC for isolates was examined by the agar dilution method. Vancomycin was added to autoclaved MHA cooled to 50 ^{o}C to the final concentration from 1 to 1024 µg/ml by tow fold diluting, and the medium were dispensed to 20 µl after gently mixing. The isolates suspension, which was adjusted to 10^4 CFU/ml, was applied to the surfaces of the agar plates containing a series of concentrations of antibiotic with a steers replicator device that delivered about 3 µl of suspension. The MIC was defined as the lowest concentration of vancomycin inhibiting visible growth after 24 hours incubation at 37 ^{o}C. Isolates were described either as resistant (by MIC ≥16 µg/ml), intermediately resistant (by MIC=4-8 µg/ml) and or sensitive (by MIC ≤2 µg/ml) [4], [14] and [15].

DNA extraction

20 µl of Tissue buffer (0.25% SDS + 0.05 M NaOH) was poured into the microtube, the amount of pure bacterial colonies were solved within. Then microtube was placed for 10 min at 95 ^{o}C, was centrifuged at 13000 rpm for 1 min, and 180 µl deionized water was added into the microtube.

PCR

The presence of the vanA gene was verified by means of PCR. The PCR amplification contained the following components: 1.5 mM $MgCl_2$, 200 µl each dNTP, 1µl each primer, 2 µl extracted DNA, 3% (v/v) DMSO, 1U phusion DNA polymerase. The amplification conditions were initial denaturation at 97 ^{o}C for 2 min, followed by 35 cycles of denaturation at 97 ^{o}C for 1 min; annealing at 52 ^{o}C for 55 s; polymerization at 72 ^{o}C for 1 min 30 s, and final extension at 72 ^{o}C for 10 s [16]. Then PCR product was assessed in a agarose gel 2% .

RESULTS

Of the 186 meat samples analyzed 46 (24.73%) were contaminated with *S. aureus*. 19 (21.34%) of the 89 meat chicken samples, 10 (33.33%) of the 30 meat turkey samples, 11 (23.40%) of the 47 meat cow samples and 6 (30%) of the 20 meat sheep samples were contaminated with *S. aureus* (Table 2).

In the vancomycin resistance tests of 46 strains isolated, VRSA were detected within 24 strains (52.17%), 9 strains (19.57%) VISA and 13 strains (28.26%) VSSA were also identified (Table 3).

Table 1. Used primers in this study

Primer sequence (5'-3')	PCR product Size	Reference
vanA Forward 5'- AAT ACT GTT TGG GGG TTG CTC -3'	734 bp	13
vanA Revers 5'- CTT TTT CCG GCT CGA CTT CCT -3'	734 bp	13

Table 2. Occurrence of *S. aureus* in analyzed meat samples

Analyzed samples	Number of samples	Number of isolates
Chicken	89	19 (21.34%)
Turkey	30	10 (33.33%)
Cow	47	11 (23.40%)
Sheep	20	6 (30%)
Total	186	46 (24.73%)

Table 3. Results of susceptibility antibiotic test

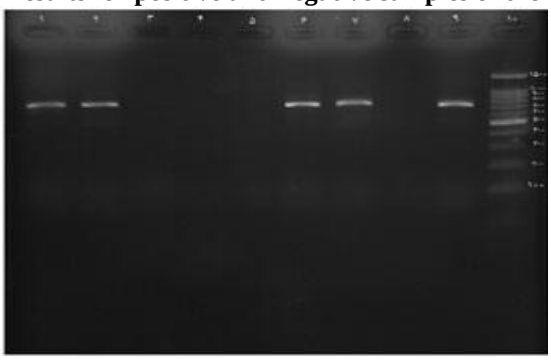
Analyzed samples	Resistant	Intermediate	Susceptible
Chicken	8	4	7
Turkey	6	2	2
Cow	7	2	2
Sheep	3	1	2
Total	24 (52.17%)	9 (19.57%)	13 (28.26%)

Among all isolated strains, 16 strains (34.78%) were detected as containing the vanA gene (Table 4).

Table 4. Number of isolates of containing the vanA gene

Analyzed samples	Number of isolates of containing the vanA gene		
	R	I	S
Chicken	3	2	1
Turkey	3	-	-
Cow	5	-	-
Sheep	2	-	-
Total	16 isolates		

Fig 1. PCR results for positive and negative samples of the vanA gene



10) DNA Ladder RTU 1500 bp. 9) Positive vanA sample as control (734bp). 8) Negative vanA sample as control. 1,2,6,7) Positive vanA samples extracted from the isolates. 3,4,5) Negative vanA samples extracted from the isolates.

DISCUSSION AND CONCLUSION

Staphylococcus aureus is well established as a clinical and epidemiologicalpathogen: in this study it was demonstrated that the potentially pathogenic role of *S. aureus* as a food-borne pathogen should not be neglected. Antibiotic-resistant isolates might be transmited to humans by the consumption of food products containing such resistant and multiresistant bacteria and that the use of antibiotics as growth promoters in animal husbandry, especially of those commonly used for broth human and animal care, should be avoided. Food is an important factor for the transfer of antibiotic resistances. Such transfer can occur by means of antibiotic residues in food, through the transfer of resistant food-borne pathogens or through the ingestion of resistant strains of the original food microflora and resistance transfer to pathogenic microorganisms.

In this study we describe the isolation and vancomycin susceptiblility characterization of *S. aureus* from chicken, turkey, cow and sheep meats. Our results indicate the 24.73% of samples were positive for *S. aureus*. 52.17% of isolates as VRSA and 19.57% of isolates as VISA were identified.

Andrew E. Waters *et al.,* in 2011, were characterized the prevalence, antibiotic susceptibility profiles and genotypes of *S. aureus* among US meat and poultry samples (n=136). *S. aureus* contaminated 47% of samples, and multidrug resistance was common among isolates (52%). One strain from isolates was resistant to vancomycin [1]. Otalu Otalu Jr *et al.,* in 2011, a total of 400 sample were collected from 200 live chickens and 200 slaughtered chickens and examined for the presence of *S. aureus*. The susceptibility of 13 *S. aureus* isolates to 12 antimicrobials was determined by disk diffusion method according to CLSI standards. 46.2% isolates had resistance to vancomycin [17]. Zhang Y. *et al.,* in 2011, a total of 280 *S. aureus* samples were used. 87 *S. aureus* isolates were obtained from suspected food samples (meat and milk) and 84 isolates were collected from food poisoning. The susceptibilities of these *S. aureus* strains to 11 antibiotics were determined. However, the presentage of *S. aureus* strains separated from food which had antibiotic resistance was 59.77. The strains isolated from food samples only showed resistance to rifampicin, vancomycin, levofloxacin and nitrofurantoin [18]. Gundogan N. *et al.,* in 2003-2004, 150 samples of raw calf/lamb meat samples and chicken parts were analyzed for presence of *S. aureus*. 80 *S. aureus* strains were isolated. The overall methicillin resistance rate was 67.5% of S.aureus strains. All strains were susceptible to vancomycin [19]. Dresses Daka *et al.,* in 2012, 160 milk samples were screened for the presence of *S. aureus* and antibiotics resistance. All the samples were contaminated with *S. aureus*. A total of 78 *S. aureus* isolates were obtained during this study. 38.5% of strains were resistant to vancomycin [20]. Stefano Morandi *et al.,* in 2009, 122 *S. aureus* isolates were collected from different dairy products. Moreover, strain resistance to vancomycin and methicillin (oxacillin) was studies. Of 122 strains studies 120 were sensitive to vancomycin while the other 2 strains showed, according to NCCLS, intermediate resistance to this antibiotic. None of the strains isolated from dairy products showed resistance to methicillin [21]. Sasidharan S. *et al.,* in 2011, were reported that of 50 dairy products samples examined, 5 (10%) were contaminated with *S. aureus*. Susceptibility, the 5 isolates were subjected to antimicrobial resistance pattern using five antibiotic disks (methicillin, vancomycin, kanamycin, chloramphenicol and tetracycline). One sample showed resistance to methicillin and vancomycin. One sample showed intermediate response to tetracycline. The other samples were susceptible to all the antibiotics tested [22]. In study of Sina H *et al.,* in 2011, 160 food samples were experimented for presence of *S. aureus*. About 56.25% of

food dishes analyzed were contaminated. 15.18% of *S. aureus* were resistant to methicillin. All isolated bacterial colonies are resistant to penicillin G. However, they were all sensitive to vancomycin [23]. Pereira V. *et al.,* in 2006-2008, a total of 148 *S. aureus* strains isolated from different food origins were identified to the species level. 10% of isolates were shown intermediate resistance to vancomycin [3].

In conclusion, these findings highlight the high potential risk for consumers in the absence of strict hygienic and preventative measures to avoid the presence of *S. aureus* isolates and resistant gene to vancomycin in raw meat, emphasizing the need for improved hygiene practices during food processing and also during the distribution and consumption of the final food products.

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REFERENCES

1. Waters AE *et al.,* Multidrug-Resistant *Staphylococcus aureus* in US Meat and Poultry. *Clinical Infection Diseases Advance Access published.* 52(10), 2011, 1-4.
2. Normanno G *et al.,* Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *International Journal of Food Microbiology*, 115, 2007, 290-296.
3. Pereira V *et al.,* Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. Escola superior de Biotecnologia-Universidade Catolica Portuguesa, Rua Dr. Antonio Bernardino de Almeida, 2008. 4200-072 Porto, 278-282.
4. Heo HJ *et al.,* Antimicrobial resistance of *Staphylococcus aureus* isolated from domestic and imported raw meat in Korea. *Korean J Vet Res*, 48(1), 2008, 75-81.
5. Ombui JN, Kimotho AM and Nduhiu JG. Antimicrobial Resistance Patterns and Plasmid Profiles of *Staphylococcus aureus* Isolated from Milk and Meat. *East African Medical Journal*, 77, 2000, 463-467.
6. Tomatari FH *et al., Helicobacter pylori* resistance to metronidazole and clarithromycin in dyspeptic patients in Iran. *Iranian Red Crescent Medical Journal*, 12(4), 2010, 409-412.
7. Yasliani S *et al.,* Linezolid vancomycin resistant *Enterococcus* isolated from clinical samples in Tehran hospitals. *Indian Journal of Medical Sciences*, 63, 2009, 297-302.
8. Brown T *et al.,* Prevalence of MRSA and Antimicrobial Resistance of *Staphylococcus aureus* in Maryland Ground Meat Products. Department of Nutrition and Food Science, 2010, 1-147.
9. Simeoni D *et al.,* Antibiotic resistance genes and identification of *Staphylococci* collected from the production chain of swine meat commodities. *Food Microbiology*, 25, 2008, 196-201.
10. Aleyasin A *et al.* Resistance to vancomycin in *enterococcus faecium* and faeca lis clinical isolates. *Pak J Med Sci*, 23(3), 2007, 390-393.
11. Ashraf R and Shah NP. Antibiotic resistance of probiotic organisms and safty of probiotic dairy products. *International Food Research Journal, Australia*, 18(3), 2011, 837-853.
12. CLSI. Performance Standards for Antrmicrobial Susceptibility Testing; Seventeenth Informational Supplement. Clinical and Laboratory Standards Institute (CLSI) 26-27, 2007, 1-182.
13. Coyle MB. Manual of Antimicrobial Susceptibility Testing. American society for Microbiology, 2005, 1-241.
14. EUCAST. Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Europian Committee for Antimicrobial Susceptibility Testing (EUCAST) of the Europian Society of Clinical Microbiology and Infectious Diseases (ESCMID), 6, 2000, 509-515.
15. OIE, Laboratory Methodologies For Bacterial Antimicribial Susceptibility Testing. NB: Version adopted by the World Assembly of Delegates of the OIE. OIE Terrestrial Manual, Guideline 4.1, 2012, 1-11.
16. Khan SA *et al.,* Molecular characterization of multidrug-resistant *Enterococcus spp*, from poultry and dairy farms: detection of virulence and vancomycin resistance gene *markers by PCR. Mol cell Probes*, 19(1), 2005, 27-34.
17. Otalu JR *et al.,* Multi-drug resistant coagulase positive *Staphylococcus aureus* from live and slaughtered chickens in Zaria, Nigeria. *International Journal of Poultry Science*, 10(11), 2011, 871-875.
18. Zhang Y *et al.,* Molecular analysis and antibiotic resistance investigation of *Staphylococcus aureus* isolates associated with *Staphylococcal* food poisoning and nosocomial infections. *African Journal of Biotechnology*, 10(15), 2011, 2965-2972.
19. Gundogan N *et al.,* A note on the incidence and antibiotic resistance of *Staphylococcus aureus* isolated from meat and chicken samples. *Department of Biology*, 69(4), 2004, 1-4.
20. Daka D, Silassie SD and Yihdego D. Antibiotic-resistance *Staphylococcus aureus* isolated from cow,s milk in Hawassa area, south Ethiopia. *African Journal of Microbiology Research*, 6(27), 2012, 5618-5624.
21. Morandi S *et al.,* Phenotypic and Genotypic Characterization of *Staphylococcus aureus* strains from Italian dairy products. *International Journal of Microbiology*, 501362, 2009, 1-7.
22. Sasidharan S, Prema B and Yoga Latha L. Antimicrobial drug resistance of *Staphylococcus aureus* in dairy products. *Asian Pacific Journal of Tropical Biomedicine*, 1(2), 2011, 130-132.
23. Sina H *et al.,* Characterization of *Staphylococcus aureus* isolated from street foods: Toxin profile and prevalence of antibiotic resistance. *Journal of Applied Biosciences*, 46, 2011, 3133-3143.