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Research Article

Prevalence of Integrons among Food Borne Bacteria and their Relationship with Antimicrobial Resistance in West of Iran

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Abstract

Background: Integrons are considered to play an important role in the spread of antibiotic resistance.

Objective: The aim of this study was to determine the distribution of integron-mediated antibiotic resistance in bacterial strains commonly found in Animal Originated Foods in Iran/Hamedan.

Methods: A total of 68 strains of *S. aureus* and 47 strains of *Enterococcus* spp were characterized for integron content and for resistance to antibiotics. The presence of *van* genes and *mecA* were investigated using PCR assay.

Results: Thirty isolates carried class 1 (*intI1*) integrons and two isolates carried class 2 (*intI2*) integrons in *S. aureus*. The frequency of class 1 in *Enterococcus* spp was fifty nine and no class 2 integron was detected. One resistance gene arrays were identified among the class 1 integrons (aadA1 cassette). These results indicate that integrons are widespread among isolates of food borne *Enterococcus* spp and *S. aureus* isolates.

Conclusion: In Iran, Due to the indiscriminate use of antibiotics and Poor performance of regulations on the clinical and food animals use of antibiotics, antibiotic resistance is increasing. This is the first report of integrons in food borne *Enterococcus* spp and *S. aureus* isolates in Iran.

Keywords: *Integrons; Enterococcus spp; S. aureus; Antibiotic Resistance*

Introduction

Indiscriminate use of the antibiotics in veterinary for treating a wide range of bacteria causing infections in animals leads to the emergence antibiotic resistance. The emergence of antimicrobial resistance has become a major problem that will result in increased mortality and mobility. During the last decades, Antibiotic resistance in bacteria through food chain has been increased and be regarded by researchers. Actually, these resistant bacteria can be transmitted to human bacterial pathogens [1]. Examples of common food - borne pathogens include *Salmonella* spp, *L. monocytogenes, E. coli O157, V. parahaemolyticus, Clostridum botulinum, Campylobacter* and toxins produced by *Staphylococcus aureus* which can cause serious complications, including death [2-4]. Genes encoding antibiotic resistance are often associated with plasmids, transposons and integrons. Integrons are a gene capture and expression system known to be responsible for multidrug resistance. They consist of an integrase gene (*intI*), which encodes an integrase (a recombination site), a receptor site called *attI* which is recognized by the integrase located proximal to the integrase gene and a promoter region (PC) that directs transcription of antibiotic resistance genes in the integrated cassettes of integrons [5]. Widespread of integrons in animal bacterial isolates have shown only in few studies [6,7]. To date, at least nine classes of integrons were isolated which Class 1 integrons are the most prevalent in both animal and human clinical bacterial isolates. Due to increasing the risk of transmission and spread of antibiotic-resistant pathogens from animals to humans, this study determine the distribution of integron-mediated antibiotic resistance in a genus of bacterium commonly found in Animal Originated Foods.

Methods

Bacterial strains: A total of 68 strains of *S. aureus* and 47 strains of *Enterococcus* spp (Table 1) were collected from the rural areas located in Hamedan Province, West Iran, from September 2015 to June 2016. All samples submitted at the microbiology laboratories of Hamedan University and were identified by standard biochemical methods. Finally all of isolates verifying by *nuc, ddl E.faecalis, ddl E.faecium, avi* and *gall* genes to identify *S. aureus, E. faecalis, E.faecium, E. avium* and *E. gallinarum* respectively.

	Raw Chicken Meat (n.)	Raw Milk (n.)	Dairy Product (n.)	Raw beef Meat (n.)
Entrococci Spp (47)	*E. faecium* (8) *E. faecalis* (4)	*E. faecium* (6) *E. faecalis* (7)	*E. faecium* (1) *E. faecalis* (4)	*E. faecium* (5) *E. faecalis* (8) *E.avium* (3) *E. gallinarum* (1)
S. aureus (68)	10	39	19	-

Table 1: *Food born isolates used in this study.*

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (MAST, Merseyside, UK). *E. faecalis ATCC 29212* (Vancomycin sensitive), *E. faecalis ATCC 51299* (Vancomycin resistance), *E. faecalis E206* (Vancomycin resistance) and *S. aureus ATCC 25423* strains were used for quality control.

DNA extraction

Template DNA of *S. aureus* and *Enterococcus* spp were prepared by using BioFlux Co., Japan Kit according to the manufacturer's instruction and boiling, respectively.

Integron characterization and sequencing of resistance encoding gene cassettes

The presence of integrons genes and resistance encoding gene cassettes associated with class 1 was investigated by PCR using specifics primers (Table 2). The PCR was performed in a reaction mixture with total volume of 25 µl, containing 2 µl template DNA; 0.2 mM of each deoxynucleoside triphosphate; 10 pmol of each primers; 10 mM Tris- HCl; 1.5 mM $MgCl_2$; 50 mM KCl; 1.5 U of Taq DNA polymerase. PCR was performed with the Eppendorf and Biorad thermocycler (ASTEC Co., Japan). Amplification was done as follows: initial denaturation step at 94°C for 5 minute followed by 35 cycles consisting of denaturation (94°C for 30 seconds), annealing (55°C, 30 seconds for *intI1*, *intI2* and 5'CS and 3'CS), and extension (72°C for 1 minute), followed by a final extension step. The PCR products of variable regions were cut out from the agarose gel, purified by QIAquick gel extraction kit (Qiagen, Valencia, CA, USA) and Sequencing of gene cassettes of class1 integron was performed with the ABI 3730X capillary sequencer (Genfanavaran, Macrogen, Seoul, Korea). Finally, Sequence data were analyzed with Chromas software and aligned with the GenBank databases by using the BLAST algorithm, which is available through the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

Gene targets	Primer sequences (5' to 3')	Amplicon/product size (bp)	References
Nuc	F: GCGATTGATGGTGATACGGTT R: AGCCAAGCCTTGACGAACTAAAGC	279	8
ddl E. faecalis	F: ATCAAGTACAGTTAGTCTTTATTAG R: ACGATTCAAAGCTAACTGAATCAGT	941	9
ddl E. faecium	F: TTGAGGCAGACCAGATTGACG R: TATGACAGCGACTCCGATTCC	658	9
Gall E.gallinarum	F: GAAAGACAACAGGAAGACCGC R: TCGCATCACAAGCACCAATC	158	10
AviE.avium	F: CGGGGAAGATGGCAGTAT R: CGCAGGGACGGTGATTTT	229	10
vanA	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	732	11
vanB	F: ATGGGAAGCCGATAGTC R: GATTTCGTTCCTCGACC	635	11
vanC	F: AGCAATAAATCTTTGTGGGTTCGT R: ATTTGCGGCAATGAAAGACAG	158	10
vanD	F: TGTGGGATGCGATATTCAA R: TGCAGCCAAGTATCCGGTAA	500	11
mecA	F: TCCAGATTACAACTTCACCAGG R: CCACTTCATATCTTGTAACG	162	12
intI1	F:CAGTGGACATAAGCCTGTTC-3' R:CCCGACGCATAGACTGTA-3'	160	13
intI2	F:TTGCGAGTATCCATAACCTG-3' R:TTACCTGCACTGGATTAAGC-3'	288	13
3CS, 5CS	F:GGCATCCAAGCAGCAAG R: AAG CAG ACT TGA CCT GA	Variable	14

Table 2: *Primers used in this study.*

Detection of *van* and *mecA* genes by PCR method

The presence of *vanA, vanB, vanC* and *vanD* genes for *Enterococcus* spp and *mecA* gene for *S. aureus* was detected by the PCR using specifics primers (Table 2). The PCR mixtures with a final volume of 25 ml consisted of template DNA (2 µl for *mecA* gene and 3 µl for other genes); 0.2 mM of each deoxynucleoside triphosphate; 10 pmol of each primer; 10 mM Tris-HCl; 1.5 mM $MgCl_2$; 50 mM KCl; and 1.5 U of Taq DNA polymerase. Amplification involved an initial denaturation at 94°C, 3 minutes followed by 30 cycles of denaturation (94°C, 1 minute), annealing (57°C and 54°C for *mecA* gene and the other genes, respectively, 1 minute) and extension (72°C, 1 minute), with a final extension step (72°C, 7 minutes).

Results

Susceptibility of *S. aureus* and *Enterococcus* spp isolates to antimicrobial agents

Antimicrobial susceptibilities data for *S. aureus* and *Enterococcus* spp isolates are presented in tables 3 and 4. The highest rate of resistance among all *intI1*-positive and *intI1*-negative isolates in *Enterococcus* spp isolates, showed against tetracycline, erythromycin and also vancomycin in *intI1*-negative isolates. While in *S. aureus* isolates showed against Erythromycin, tetracycline in all *intI1*-positive and *intI1*-negative isolates and Gentamicin in *intI1*-positive isolates.

Antimicrobial agent	Integron-positive isolates (n = 28)			Integron-negative isolates (n = 19)			Total (n = 47)		
	S	I	R	S	I	R	S	I	R
Cloramphenicol (30 µg)	23	3	2	17	1	1	40	4	3
Nitrofurantoin (300 µg)	24	3	1	19	0	0	43	3	1
Linezolid (30 µg)	24	4	0	17	0	2	41	4	2
Ampicillin (10 µg)	16	0	12	15	0	4	31	0	16
Vancomycin (30 µg)	9	5	14	5	6	8	14	11	22
Teicoplanin (30 µg)	25	3	0	14	5	0	39	8	0
Tetracycline (30 µg)	10	0	18	10	1	8	20	1	26
Ciprofloxacin (5 µg)	4	8	16	4	9	6	8	17	22
Norfloxacin (10 µg)	5	11	12	3	11	5	8	22	17
Erythromycin (15 µg)	2	12	14	2	7	10	4	19	6

Table 3: *Antimicrobial susceptibility of integron-positive and integron-negative of Enterococcus spp.*

Antimicrobial agent	Integron-positive isolates (n = 32)			Integron-negative isolates (n = 36)			Total (n = 68)		
	S	I	R	S	I	R	S	I	R
Cefoxitin (30 µg)	22	0	10	25	0	11	47	0	21
Erythromycin (15 µg)	18	1	13	22	2	12	40	3	25
Tetracycline (30 µg)	19	0	13	23	0	13	42	0	26
Gentamicin (30 µg)	19	0	13	26	0	10	45	0	23
Clindamycin (2 µg)	22	0	10	30	0	6	52	0	16
Ciprofloxacin (5 µg)	20	0	12	26	0	10	46	0	22
Rifampin (5 µg)	23	0	9	26	0	10	49	0	19
Trimethoprim-Sulfamethoxazole (SXT) (1.25/23.75 µg)	27	0	5	32	0	4	59	0	9

Table 4: *Antimicrobial susceptibility of integron-positive and integron-negative of S. aureus.*

Analysis of integrons

Among the total 68 isolates of *S. aureus*, 32 (47%) isolates and 2 (2.9%) isolates carried class 1 (*intI1*) or class 2 (*intI2*) integrons, respectively. The frequency of class 1 in *Enterococcus* spp was 59.5% (28/30). No class 2 integron was detected. Sequence analysis of in-

tegron's variable region indicated that the presence of aminoglycoside 3'-adenyltransferase (*aadA1*; 1000 bp), gene cassettes among the isolates, which 31.2% (10/32) of *S. aureus* isolates and 89.2% (25/28) of *Enterococcus* spp harboring *aadA1* gene cassettes.

Presence of *van* and *mecA* genes

According to the PCR assay for *van* and *mecA* genes, Twenty-two and four isolates of *Enterococcus* spp were positive for *vanA* and *vanB* respectively. 14 (63.6%) isolates of *vanA* gene carried class I integrons. One isolates was positive for *vanC* (*E. gallinarum*). No vanD-positive isolates were detected. In addition, 4 isolates were positive for *vanA* and *vanB*. 17 isolates of *S. aureus* were positive for *mecA* and 8 isolates were positive for *mecA* and class I integrons.

Discussion

For many decades, antibiotic resistance is one of the world's most pressing public health problems. Various scientific studies has been shown that more than half of all commercial antibiotics are used in food-producing animals and there is a link between the use of antibiotics in during agricultural production and antimicrobial resistance of human pathogens [15-18]. In fact, Genes encoding antibiotic resistance can be transfer by the horizontal transfer of genetic elements such as plasmids, transposones and integron. And in this way, antibiotic-resistant food borne bacteria, such as *S. aureus, E. coli O157, V. parahaemolyticus, Salmonella* spp. and *L. monocytogenes* can be transmitted to humans. There are little documents on the antibiotic resistance of enterococci and staphylococcus strains existing in foods, while strains from clinic specimens have been widely investigate. Therefore, the present study reports antibiotic resistance profile of food borne pathogens in retail meats and dairy products were sold commercially in Hamedan, west Iran. In addition we determined Integrons among them. During the study period, a total of 730 food samples including 466 of meat samples and 264 of milk and dairy product samples were isolated. Among these samples, *S. aureus* strains were isolated in 58 of meat samples and 10 samples of milk and dairy product samples. While A total of 47 Enterococci (including 20 meat samples and 17 milk and dairy product samples) were isolated from 70 food samples. Observations of the present study showed that, the prevalence *S. aureus* strains in meat samples are much less than other samples. Similar to results of Ammar., *et al.* in Egypt [19]. Our results contrast with the studies by Angkititrakul., *et al.* in Thailand [20]. Among the total 47 isolates of *Enterococcus* spp, *E. faecalis* (48.9%) was the most prevalent and *E. gallinarum* (2.1%) was the lowest rate which the results is similar to Chajecka., *et al*. in Poland [21]. The most common species were *E. faecium* in raw meat and *E. faecalis* in milk and dairy products. The results are contrast with the studies by Pesavento., *et al.* in Itay [22]. The highest rate of resistance among all *intI1*-positive and *intI1*-negative isolates, showed against erythromycin and tetracycline in *S. aureus* strains and of *Enterococcus spp* which is similar to results obtained in previous study in clinical samples in Hamedan [23]. In this study, we found that a large percentage of *Enterococcus* spp and *S. aureus* strains harbored integrons, the class 1 integron being the most prevalent. Whilst none of Vancomycin-resistant *Staphylococcus aureus* (VRSA) investigated in Ammar et, harboured class 1 integrons [19]. We also observe one gene cassette in *IntI1* positive isolates, including aadA1 which was encoded protein may contribute to the bacterial isolates' resistance to aminoglycosides (streptomycin/spectinomycin). In our study, the class 1 integron containing the *aadA1* gene was similar to that found in Gram-negative and Gram positive bacteria [23-24]. This could indicate the transfer of resistance genes that may occur between food borne bacteria and human bacterial pathogens. Simultaneous presence of van genes and *mecA* with class 1 integrons enhances possibility of spreading antibiotic resistance via horizontal gene transfer. In Iran, Due to the indiscriminate use of antibiotics and poor performance of regulations on the clinical and food animal's use of antibiotics, antibiotic resistance is increasing.

Conclusion

In accordance to the results of this study, the widespread occurrence of integrons proved to be a major challenge for the treatment and control of infectious diseases. This is the first report of integrons in food borne *Enterococcus* spp and *S. aureus* isolates in Iran.

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Conflict of Interest

All the authors declare that there is no conflict of interest in this study.

Bibliography

1. Sunde M. "Prevalence and characterization of class 1 and class 2 integrons in Escherichia coli isolated from meat and meat products of Norwegian origin". *Journal of Antimicrobial Chemotherapy* 56.6 (2005): 1019-1024.

2. Xu Z., *et al*. "Occurrence and characteristics of class 1 and 2 integrons in Pseudomonas aeruginosa isolates from patients in Southern China". *Journal of Clinical Microbiology* 47.1 (2009): 230-234.

3. Xu Z., *et al*. "Nosocomial infection caused by class 1 integron-carrying Staphylococcus aureus in a hospital in South China". *Clinical Microbiology and Infection* 13.10 (2007): 980-984.

4. Wang L., *et al*. "Development and application of a simple loop-mediated isothermal amplification method on rapid detection of Listeria monocytogenes strains". *Molecular Biology Reports* 39.1 (2012): 445-449.

5. Koczura R., *et al*. "Association between the presence of class 1 integrons, virulence genes, and phylogenetic groups of Escherichia coli isolates from river water". *Microbial Ecology* 65.1 (2013): 84-90.

6. Bass L., *et al*. "Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian Escherichia coli". *Antimicrobial Agents and Chemotherapy* 43.12 (1999): 2925-2929.

7. Martinez-Freijo P., *et al*. "Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds". *Journal of Antimicrobial Chemotherapy* 42.6 (1998): 689-696.

8. Hajiahmadi F., *et al*. "Assessment of the Prevalence of Class I and II Integrons of Escherichia coli and Klebsiella pneumoniae Isolates from Hospitals of Hamadan". *Scientific Journal of Hamadan University of Medical Sciences* 23.3 (2016): 193-201.

9. Hosseini M., *et al*. "Presence of virulence factors and antibiotic resistances in Enterococcus sp collected from dairy products and meat". *Der Pharmacia Lettre* 8.4 (2016): 138-145.

10. Zhang K., *et al*. "New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of Staphylococcus aureus from coagulase-negative staphylococci". *Journal of Clinical Microbiology* 42.11 (2004): 4947-4955.

11. Skaltsa HD., *et al*. "Essential oil analysis and antimicrobial activity of eight Stachys species from Greece". *Phytochemistry* 64.3 (2003): 743-752.

12. Dutka-Malen S., *et al*. "Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR". *Journal of Clinical Microbiology* 33.1 (1995): 24-27.

13. Chen B., *et al*. "Class 1 integrons, selected virulence genes, and antibiotic resistance in Escherichia coli isolates from the Minjiang River, Fujian Province, China". *Applied and Environmental Microbiology* 77.1 (2011): 148-155.

14. Rowe-Magnus DA., *et al*. "Integrons: natural tools for bacterial genome evolution". *Current Opinion in Microbiology* 4.5 (2001): 565-569.

15. Mathur S., *et al*. "Antibiotic resistance in food lactic acid bacteria—a review". *International Journal of Food Microbiology* 105.3 (2005): 281-295.

16. Doublet B., *et al*. "Variant Salmonella genomic island 1 antibiotic resistance gene cluster containing a novel 3′-N-aminoglycoside acetyltransferase gene cassette, aac (3)-Id, in Salmonella enterica serovar Newport". *Antimicrobial Agents and Chemotherapy* 48.10 (2004): 3806-3812.

17. Acar J., *et al*. "Antimicrobial resistance: an overview". *Revue Scientifique et Technique-Office International des Epizooties* 20.3 (2001): 797-807.

18. Depoorter P., *et al*. "Assessment of human exposure to 3rd generation cephalosporin resistant E. coli (CREC) through consumption of broiler meat in Belgium". *International Journal of Food Microbiology* 159.1 (2012): 30-38.

19. Ammar A., *et al*. "Class 1 integron and associated gene cassettes mediating multiple-drug resistance in some food borne pathogens". *International Food Research Journal* 23.1 (2016): 1-7.

20. Angkititrakul S., *et al*. "Prevalence of Salmonella enterica, Escherichia coli and Staphylococcus aureus in Raw Meat in Thai Self-Service Style Restaurants in Khon Kaen Municipality". *The Thai Journal of Veterinary Medicine* 43.2 (2013): 265-271.

21. Chhajeckae-Wierzchowska W., *et al*. "Diversity of antibiotic resistance genes in enterococcus strains isolated from ready to eat meat products". *Journal of Food Science* 81.11 (2016): 2799-08.

22. Pesavento G., *et al*. "Prevalence and antibiotic resistance of Enterococcus spp. isolated from retail cheese, ready-to-eat salads, ham, and raw meat". *Food Microbiology* 41.1 (2014): 1-7.

23. Hajiahmadi F., *et al*. "Prevalence of class 1 and 2 integrons in Enterococcus spp. and their relationship with antimicrobial resistance". *Journal of Chemical and Pharmaceutical Sciences* 9.3 (2016): 1368-1373.

24. Hajiahmadi F., *et al*. "The frequency of integrons of antibiotic resistant in Stenotrophomonas maltophilia isolates in Hamadan/Iran". *Iranian Journal of Medical Microbiology* 10.4 (2016): 10-16.

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