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Antibiotic Susceptibility Pattern among Vancomycin Resistant Enterococci Isolated from Meat and Fecal Samples in Tehran Livestock Husbandries

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Enterococci are part of the normal flora of humans and animals. Recently, Enterococci have caused great concern due to developing of resistance to many antimicrobial agents. Their ubiquity in animal and human digestive tracts , medical importance, frequent multiple antibiotic resistance and their seemingly unlimited capacity for horizontal gene transfer via numerous mobile genetic elements make this bacterial group ideal for investigating the ecology of antibiotic resistance. The aim of this study was to investigate and identify the prevalence of VRE (Vancomycin Resistant Enterococcus) within isolated Enterococci taken from a number of Tehran Livestock husbandry units. Putative Enterococci (n=242) were isolated on Membrane Filter Enterococcus Selective Agar Medium , supplemented with 2 , 4 and 8 μgr/ml vancomycin from cow samples. A total isolates passed the standard biochemistry tests for the genus and species as well as specific genus and species primers. The antibiotic susceptibility was determined by the disc diffusion method for 6 antibiotics. MIC of vancomycin was also done using broth micro-dilution assay by CLSI recommendations. Results showed that 138, 90 and 14 of the isolates were E. faecium , E. feacalis and E. gallinarum, respectively. 41, 25, 18, 10, 21 and 22 of the isolates were resistant to vancomycin , tetracycline, gentamicin , chroramphenicol, ciprofloxacin and erythromycin, respectively. An MIC test on 65% of the isolates was >256 μgr/ml. Diversity of VRE isolates was restricted to 3 species. E. faecium had high resistance to a broad range of antibiotics. The results of this study suggest that more attention should be paid to the livestock samples as a reservoir of resistance elements. Surveillance of VRE reservoirs in animal husbandry to clarify the mechanism of transfer are urgently required.

Key Words: Enterococcus, livestock, VRE, Tehran.

Enterococci are gram positive, opportunistic bacteria that inhabit the gastrointestinal tracts of human and many animals. They can be found in the upper respiratory tract, mouth, vagina and perineum[1-4]. They can also frequently colonize a variety of lesions including cutaneous ulcers and wounds . Over the last few years, *Enterococci have become more important* as nosocomial pathogens . Their intrinsic or acquired resistance to many antibiotics, in particular, to glycopeptides, has become a major concern[5,6]. These organisms were known to cuase endocarditic and urinary tract infections by the early 1900s and members of the species *E. faecalis* were known to be a common cause of infections[1,4]. The emergence of *Enterococci* as important

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pathogens was an unwelcome therapeutic challenge for clinical medicine and microbiology. Vancomycin Resistant *Enterococci* or VRE are the main sources of infections in humans and animal and carriers of transferable vancomycin resistance marks[8,7]. Vancomycin is considered to be one of the antibiotics of last resort when most of the antibiotics fail in treating the infections caused by gram positive bacteria[11,12]. *E. faecalis* and *E. faecium* are generally the most common enterococcal species in the animal and human intestinal flora and thus also the most common species in hospital and urban waste waters[7,8]. *Enterococci* are also present in food, as starter cultures for the production of cheese and fermented sausages or as fecal contaminant of raw meat, milk and milk products[10] . Several lines of evidence have supported a link between the use of glycopeptides antibiotic avoparcin as a growth- promoting agent (AGPs) in livestock[9,10]. The use of avoparcin as an AGPs has been suggested as a risk factor for carriage of VRE in food animals, as the usage of any antibiotic produces a selective pressure in favor of resistance . The avoparcin has given us a unique opportunity for large-scale monitoring of the population dynamics of resistant bacteria and the fate of antibiotic resistance elements . Since avoparcin, vancomycin and their analogs are not used in animal farms, information is available on decrease of prevalence of VRE[9,10,11].

Several ecological and epidemiological studies suggest that VRE could be transmitted from animals to humans through the food chain .The present study was undertaken to investigate and identify the prevalence of VRE within isolated *Enterococci* which was separated from number of Tehran livestock and chicken husbandry unites.

MATERIALS AND METHODS

Litter samples, fecal samples and meat were collected from 6 different livestock husbandry in Tehran on the following three separate occasions over a one year period: spring 2009, summer 2009, fall 2009 and winter 2010. 242 isolates of *Enterococcus* were collected. The samples were collected in a sterile container and transported immediately after collection to the laboratory , where were analyzed no later than 3 hours . Samples were subjected to serial 10-fold dilution with normal saline, before filtration. The enumeration of *Enterococci* was performed by membrane filtration through 0.45µ pore-size membranes (Millipore Corporation, Bedford,MA,USA). The membrane filters were put on Brain Heart Infusion Agar (Becton Dickinson and Co., Sparks,MD,USA) plates and incubated for 2h at 37ºC [15-17]. The membranes were transferred to MESA (M *Enterococcus* Selective Agar) supplemented with 2, 4 and 8 µgr/ml vancomycin (St,Louis, MO). The plates were incubated at 45º for 36-48h. Typical colonies were picked from each plate and were subcultured onto BloodAgar , identified and tested for biochemical characteristic . The isolation colonies were identified by conventional methods, using tolerance to Bile , PYR positive , growth in 6.5% NaCl , absence of catalase[15,17,1,4]. Identification to the species level was performed by testing for monitol, sorbitol, methyl – alfa-glucopyranoside, motility etc. Reactions were observed after 24 h of incubation at 37ºC [13,15].

Susceptibility testing

All 242 isolates were tested for antibiotic susceptibility by the disk diffusion method. 6 different antibiotics were used that included

Table 1. Antibiotic Suscebtibility

Antibiotic	number	percentage
Erythromycin	53	20
Ciprofloxacin	48	19
Vancomycin	91	38
Tetracyclin	59	23
Chloramphenicol	38	15
Gentamicin	49	19

Table 2. Antibiotic susceptibility in VRE

Antibiotic	number	percentage
Erythromycin	34	38
Ciprofloxacin	30	33
Tetracyclin	21	23
Chloramphenicol	13	15
Gentamicin	42	46

vancomycin (30µgr), chloramphenicol (30µgr), tetracycline (30µgr), erythromycin (15µgr) , ciprofloxacin (5µgr) and gentamicin (10µgr)[16,17]. All levels were done by CLSI recommendations. The MIC for the antibiotics were determined by the broth dilution method . The concentration ranges used to determine the MIC were > 256 and >128.

RESULTS

The identification of *Enterococci* isolated from the livestock production environment did not reveal any unusual species. A total of 242 *Enterococci* were isolated from fecal and meat sample. 3 different species were determined. 9%, 36% and 41% of the isolates were *E. gallinarum* , *E. faecium* and *E. faecalis* respectively . All of the 242 isolates identified, were resistant to at least one antibiotic. The majority of *E. gallinarum* isolate were sensitive to at least 3 antibiotic including gentamicin, chloramphenicol and ciprofloxacin. The most resistance were shown to erythromycin and ciprofloxacin. Several *E. faecium* isolates were also resistant to gentamicin, specifically tetracyclin . VRE were isolated from 91 of the 242 sample studied. VRE were most commonly isolated from fecal sample. Data obtained from antimicrobial susceptibility testing summarized in Table 1. VRE were isolated from 91 of the 262 samples studied among 242 of the isolates , resistance vancomycin , erythromycin , tetracycline, chloramphenicol, gentamicin and ciprofloxacin were 41% , 22% , 25% , 10% , 18% and 21% respectively . The results of antibiotic susceptibility testing of the VRE isolates from animal sources are shown in Table 2 . High level gentamicin resistance were seen in *E. faecium* , *E. faecalis* and *E. gallinarum* respectively. The rates of resistance to erythromycin and ciprofloxacin differed for different *Enterococcus* species.

DISCUSSION

In this study, we describe the isolation , biochemical and molecular characterization of intrinsically VRE spp. from the meat and stool of cow. The results of this study shows that VRE species distribution in Iran among livestock is more variable from those taken from sewage and clinical samples. Its variety is almost the same as that of the study done by the author on sewages[15,17,4,2,1]. *E. faecium* , *E. faecalis* and *E. gallinarum* are three species, common in livestock samples. In clinical sample *E. faecalis* is more prevalent from *E. faecium* and other species. In most environmental and clinical studies without any antibiotics *E. faecium* is one of most prevalent and isolable species which are isolable. The percentages of resistance to vancomycin among the *Enterococci* isolated from the fecal sample were quite similar. The distribution of the different species in the present study differed from those described by the other researches in other countries[15,17,4,2,1]. We report here the first enterococci isolates with a high-level vancomycin resistance obtained from Iran's animal husbandry. Our results are in contrast to a recent study which reported the absence of VRE in food. In Europe, unlike the USA, VRE strains are widespread in the community , farm and pet animals. Because some antimicrobials used as feed additive have a therapeutic use in human as well[20,4,2,1]. Resistance to multiple classes of antibiotics is common in *Enterococci* as was seen in this research. The resistance rate to ciprofloxacin and chloramphenicol among *E. faecalis* was more than that to *E. faecium*. This may be because of heavy use of these 2 antimicrobials in human and animal infections and selective pressure or simply the transfer of resistance genes or a combination of both[1,4]. Studies have demonstrated a link between human source of resistant strains of *Enteroccus* isolates and the environment, and have suggested that animals and their products are contaminated secondary to interaction with humans and environment. Although the number of cases of human VRE infections in Iran's hospitals is low , the agricultural VRE reservoir that still exists may threaten the community and human medicine because of the possible transformation from *Enterococci* to *methicilin resistant S. aureus* , creating a dangerous pathogen that is difficult to treat[12]. Surveillance of the VRE reservoir in animal husbandry and further investigations to clarify the mechanisms for persistence of genes of resistance are urgently required. In comparison with other studies in Iran, on clinical and environmental species , in this study, the resistance to vacomycin is much higher . The resistance rate to vancomycin was 32% which in comparison to other study's around the world

varies. In one research on livestock , in 2005, in Vienna, VRE species detected in livestock was 21.6%. This rate for pigs and chicken was 23.3% and 77.1% [9]. In his study in 2005 , Kuhn reported the resistance to vancomycin in clinical , environmental and livestock species in Europe to be 8% to 11%[18]. Main reason for species resistance to vancomycin in Europe and USA is the high usage of Glicopeptides in clinical center and in the next step natural selection and colonization of these species in human's gastrointestinal tracts or only transferring the resistance genes to the gastrointestinal tracts resident bacteria's, while passing by and also usage of compounds like avoparcin as antimicrobial promoter[1,4,18].

Resistance to erythromycin in this study was 20% and 38% in VRE species which is a consequence of this antibiotic's vast usage in Iran. Erythromycin is also used by livestock which affects *Enterococcus* resistance[19,21]. Resistance to cyrofloxacin were 19% and 33% in VRE species. Because of its concurrence in urinary tract infections curing, this antibiotic is heavily used and like other antibiotics resistance in *E. faecium* is more probable than that of *E. faecalis* and other species. In general resistance to gentamicin was 22% and 46% in VRE species. *E. faecium* was the resistant frame to gentamicin as estimated.

Liassine and colleagues in 1998, reported the rate of 4% in species taken from clinical sewage in Switzerland. On the other hand in 2004 Khan and colleagues, of USA, reported the rate of 96% as the resistance to gentamicin[2]. Resistance to tetracycline and vancomycin in this research was 23% and 38% which shows that the resistance to tertracyclin varies a lot around the world. This rate is much too high in Switzerland , USA lower in Portugal and France[21,2].

The lowest resistance happened to be for chloramphenicol at the rate of 15%. Although this rate is bigger than the separated samples , both clinically and environmentally , in general resistance to this antibiotic is low, This may occur because of low usage according to its disinfections in vivo situation. Based on present evidence, on the one hand is this idea of horizontal VRE transmission from animal to human, and on the other hand is the various *Enterococcal* species which have the potential of duplication and stability in soil and water. This resistance to environmental factors makes the defending so difficult. Public health may face a threat from VRE transmission specially if microorganisms transmit via stool and water, because this water might be used without any treatment[1,4,13,15].

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