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Research Article

PREVALENCE OF VANCOMYCIN RESISTANT ENTEROCOCCI SPECIES IN  
MINCED BUFFALO MEAT OF CHITWAN, NEPAL

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**Abstract**

Vancomycin resistant *Enterococci* is becoming one of the concerns of public health in recent times. VRE involve those strains of *Enterococci* that are resistant to the vancomycin which is considered as last drug of choice for the Gram positive bacterial infections. Studies show enterococci to be one of the major food borne diseases and medical consequences of its occurrence in humans. Thus, a cross-sectional study was carried out to determine the prevalence of VRE in buffalo meat in Chitwan. A total of 63 retail minced meat samples were collected from different meat shops. These samples were diluted and cultured and subjected to antimicrobial susceptibility test. Of 63 samples, 16 (25.39%) samples were positive for the enterococcus species among which 3 (18.15%), were VRE indicating overall prevalence of VRE to be 4.76%. AST revealed that resistance among them was high for penicillin G and amikacin (75%) followed by tetracycline (43.75%), erythromycin (37.5%), vancomycin (18.75%) levofloxacin (12%) and methicillin. Eventhough methicillin revealed no resistance it showed intermediate sensitive to all the samples. Thus, it clarifies the presence of VRE in meat samples suggesting zoonotic disasters that can follow.

**Key words:** *Enterococci*; vancomycin; minced meat; buffalo.

**Introduction**

*Enterococci* are commonly found as a part of natural microflora of gastrointestinal tract in humans and animals (Clarence *et al.*, 2009). Till date more than forty genus of *Enterococcus* are known (Klein *et al.*, 2003) of which 12 are known to be pathogenic among which *E faecalis* and *E faecium* account upto 90% of clinical isolates (Prakash *et al.*, 2005). *Enterococci* are known to produce serious nosocomial infection causing bacteremia, urinary tract infections, endocarditis and surgical complications. In case of multidrug resistant *Enterococci* infections, vancomycin and quinupristin-dalfopristin (Synercid) are considered to be the drug of choice (McGowan *et al.*, 2006). Glycopeptide resistances in *Enterococci* that are resistant to other antibiotics produce difficulty therapeutic problems (Lukasova and Sustackova, 2003). As a result, therapeutic options for treatment of *Enterococci* infections are increasingly limited (Murray, 1990).

*Enterococci* are present in gut of animals and are naturally resistant to cephalosporins, aminoglycosides and clindamycin. They show intermediate sensitivity to penicillin, ampicillin and glycopeptides. *E faecalis* and *E faecium* isolates have shown wide spread resistant to antibiotics like chloramphenicol, macrolides kanamycin, streptomycin and tetracyclines (Ruzauskaset *et al.*, 2009). The most recent traits are reported as penicillinase resistance and vancomycin resistance which can be transferred to

other bacteria. Inappropriate use of the antibiotics increases colonization of bacteria and produces opportunity for infection and also transmits the resistant to other bacteria like *Listeria monocytogenes* and *Staphylococcus aureus* via plasmids and transposons by conjugation producing pathogenic effects in humans. High level vancomycin resistant was detected in the *E. faecalis* and *E faecium* for the first time in Europe in 1980s. Since then, VRE have been associated with nosocomial infection around the world (Leclercq *et al.*, 1988). Although nosocomial infection is being increasing the presence of food borne VRE, is yet to be documented from the meat sources in Nepal. The increasing occurrence of vancomycin resistant *Enterococci* not only possess serious problem not only in the treatment of the disease but also carries the increased risk of horizontal transfer of the resistant to other vancomycin-susceptible species (Tendolkaret *et al.*, 2003).

Buffalo meat consists of about two third of meat consumption in Nepal. Average meat shop of Nepal doesnot meet the minimum standards for hygiene and quality providing opportunity for the food borne pathogens to proliferate and infect. Major food borne pathogens include *Enterococci*, *Salmonella* and *Escherischia coli* of which, although being natural microflora in both animal and human gut. *Enterococci* are frequently responsible for the significant morbidity and mortality in predisposed man.

In context of Nepal, antibiotics are used randomly by farmers and paraveterinarians (Bhattarai, 2005). Such freedom of use of the antibiotics in animals for treatment and as probiotic has lead to propagation of the enteric organism like *Enterococci*. Transfer of such bacteria occurs via food chain and animal handlers to the human population. Thus, in our context as a mile stone data it is important to know about the prevalence of vancomycin resistant *Enterococci* from the meat.

## Methodology

### Collection and transportation of the sample

A cross sectional study was conducted in Chitwan from September to December, 2012. 63 samples of buffalo minced meat samples at least 25 grams were collected in UV treated sterile zipper plastic bags from different retail meat shops. The meat samples were placed in ice packs and were brought to microbiology lab IAAS Rampur for further microbiological analysis.

### Microbial analysis of the samples

225ml of 0.1% peptone water was transferred into the bag containing 25 gm of minced meat weighed aseptically and accurately ( $\pm 0.1$  gm). The mixture was shaken for 2 minutes and 1:10 dilution of the sample was obtained for different microbiological procedures involving cultural morphology, Gram staining, oxidase test, catalase test, 6.5% salt tolerance, motility test and hemolysis pattern observation. After above tests, the samples were preserved with 40% glycerine in the ratio 1:1 for further antibiotic sensitivity tests.

Bacterial isolates were confirmed on the basis of cultural morphology, staining and various tests. Then, these colonies were subject to antibiotic sensitivity test by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Himedia) following guidelines provided by Clinical Laboratory Standard Institute (CLSI, 2009).

### Antibiotic Sensitivity Test

Antimicrobial susceptibility testing was done by the standard Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Himedia) following guidelines provided by the CLSI (2009).

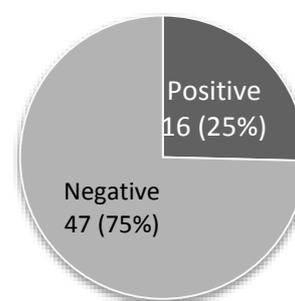
The top of each of three well isolated colonies in the nutrient agar was touched with a sterile loop and the growth was transferred into a tube containing three to four ml of a Brain-Heart Infusion Broth (BHIB). The BHIB was incubated at 35°C for 2-6 hours until it achieved the turbidity of the 0.5 McFarland standards (the turbidity of this broth culture was adjusted with sterile saline to obtain turbidity optically comparable to that of the 0.5 McFarland standards).

The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the cotton swab over the entire sterile agar surface three times, turning the plate at 60° angle between each streaking. Then drug impregnated discs (7 discs per 100mm plate) were applied using sterile forceps after allowing the inoculum to dry for 10 minutes with lid in place.

## Results and Discussion

### Prevalence of VRE in minced buffalo meat of Chitwan

Out of total 63 samples 16 were found to be positive for *Enterococcus* species (Fig. 1). Out of 63 samples 3 were positive for the VRE. The prevalence of *Enterococci* was thus found to be 25.39% and overall prevalence of VRE to be 4.76%. Of 16 positive *Enterococci* samples, 18.75% were VRE (Table. 1).



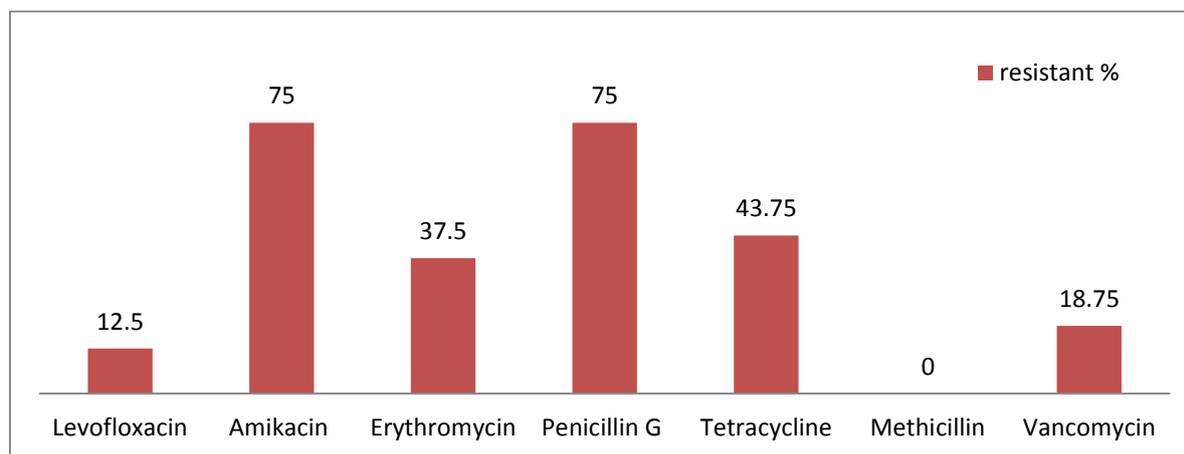
**Fig. 1:** Proportion of *Enterococcus* positive and negative samples

**Table. 1:** Prevalence of VRE in buffalo meat sample

Types of organisms	Number	Percentage (%)
Vancomycin resistant <i>Enterococci</i> (VRE)	3	4.76%
Vancomycin sensitive <i>Enterococci</i>	13	20.63%
<i>Enterococci</i> Negative Samples	47	74.60 %
Total Number of Samples	63	100%

### Antimicrobial susceptibility testing

All the species isolated were resistant to one or more antibiotics used. Highest zone of inhibition was shown by levofloxacin ( $19.44 \pm 1.34$  mm) followed by tetracycline ( $18.25 \pm 1.35$  mm), vancomycin ( $16.31 \pm 0.72$  mm) and Erythromycin ( $15.81 \pm 1.19$  mm). Methicillin ( $10.0 \pm 0.0$  mm) showed the lowest zone of inhibition. Intermediate sensitivity results were considered as sensitive as higher dose of antibiotics show them to be sensitive. Amikacin and Penicillin G were found to be most resistant (75%) to the *Enterococci* species, followed by tetracycline (43.75%), erythromycin (37.5%), vancomycin (18.75%) and levofloxacin (12.5%) (Fig. 2).



**Fig. 2:** Resistant percentage of different antibiotics used in antibiotic sensitivity test

## Discussion

This study showed that the prevalence of *Enterococci* in minced buffalo meat in Chitwan is 25.39%. The present finding is considerably lower. The prevalence of *Enterococci* was reported to be 100% (Rijal *et al.*, 2009) and Basnet *et al.* (2007) respectively. The present data also shows decline in prevalence of *Enterococci* in comparison to Hayes *et al.* (2003) who found 65% prevalence in ground beef meat and to McGowan *et al.* (2006) who found 79% on different meat samples. This reduction prevalence of bacteria in the minced meat sample could be because of the use of the samples mixed with spices and condiments. The lipophilic properties of the extract of the spices causes to lower *Enterococcus fecalis* CNRZ 238. (Sladkova *et al.*, 2011). Oonmetta-aree (2005) stated that presence of spices (ginger, turmeric, onion, peeper, etc have strong inhibiting effects on food borne pathogens.

This finding of VRE (4.76%) is in contrast to (Messi *et al.*, 2006) who reported 35% prevalence from meat samples. Moreover, this study goes along with the study by Klein *et al.* (1998) that showed lower (8.3%) prevalence in minced beef and pork meat. Rijal (2009) found no vancomycin resistant *Enterococci* in meat samples from Chitwan, Nepal. In contrast, VRE were observed in minced buffalo meat of Chitwan in this study. This might be because of increased unjustifiable use of antibiotics in animals producing resistant *Enterococci* variety in gut.

Antimicrobial susceptibility testing revealed that the *Enterococci* species were highly susceptible to levofloxacin, tetracycline and least sensitive to penicillin and methicillin. This result is in accordance to Rijal 2009 who found chloramphenicol, tetracycline, erythromycin and ciprofloxacin to be most sensitive in decreasing order followed by ampicillin. Moreover, 18.75% resistance was seen for the vancomycin and 37.5% resistance for erythromycin and 43.75% for tetracycline. In contrast, in other study by Krocko *et al.* (2007), 15% were resistant to vancomycin and 15% were resistant to erythromycin, and 56% to tetracycline. Intermediate resistance to erythromycin (48%) and tetracycline (56%) were more

prevalent. Ruzauskas *et al.*, (2009) reported resistance of 65% in tetracycline, 55% in erythromycin, 6% in vancomycin and ciprofloxacin.

## Conclusion

This study shows the prevalence of VRE in minced buffalo meat of Chitwan. This prevalence indicate the risk the meat handlers are as VRE are capable of transmitting the resistant genes to other bacteria and even produce the disease themselves creating untreatable conditions. As the bacterial is moderately heat stable, transmission to the humans from food is most likely. Levofloxacin has shown better result with least resistance for the treatment of VRE in vitro.

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