

# Epidemiological Features of Vancomycin-Resistant Enterococci in Broiler Farms in Malaysia

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## Abstract

Studies have suggested that the vancomycin-resistant enterococci (VRE) may be transferred to humans via the food chain or the environment. One of the most common source of the occurrence of VRE is the poultry. The objective of the present study is to describe the epidemiological features of VRE in terms of its prevalence and distribution in the Malaysian broiler population. A longitudinal investigation was designed where six broiler farms were sampled three consecutive times. Cloacal samples were collected when the birds were a day-old, 21-days and at 40-days old. At the time the samples were collected, environmental samples and information of the farms were also gathered. The study found that the prevalence of VRE increased as the birds matured. We also found that the prevalence of VRE is lower in a close-housing system compared to open-housing system.

## Introduction

Vancomycin-resistant enterococci (VRE) is an emerging organism in public health due to its potential for fatal infection in humans. The significance of VRE in the Malaysian local animal industry was underscored by the current demand by importing countries for VRE-free live animals and animal-derived products. This demand stemmed from suggestions that the occurrence of antibiotic multi-resistant VRE in animals might be linked to multi-resistant VRE that is causing diseases in humans (Wegener *et al*, 1999; Bonten *et al*, 2001). In Malaysia, there has not been any report of hospital-acquired VRE infection; however, one community-acquired VRE infection has been documented (Raja *et al*, 2005). The prevalence of VRE in poultry is unknown in Malaysia. A few works have been published on VRE in chicken carcasses (Radu *et al*, 2001, Ong *et al*, 2002) and eggs (Hassan *et al*, 2005). However, these studies lacked the farm epidemiological components. The present study was undertaken to estimate the prevalence of VRE over the rearing period of broiler chickens and to identify the risk factors for the occurrence of the organism at the farms.

## Materials and Methods

### Study population

Most broiler farms in Malaysia are owned and subcontracted by private companies. Therefore, it is difficult to obtain permission to enter the farms. The selection of farms for this project was ultimately based on permission by the company and willingness of the farm veterinarian and farm owners to permit samples to be collected. The farms involved in this study cater for the local market only.

### Study design

A longitudinal study design was employed to economise on subjects, as only a few farms were accessible for the project. The selected flocks of broiler chickens in the six farms (three open-house and three close-house system) were followed and sampled at intervals over a period of 6 weeks.

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Within this period, samplings in each farm were carried out as soon as the chicks arrived at the farm from the hatcheries (DOC), when the birds were 21-days and 40-days old. At each sampling, 30 cloacal swabs and five environmental samples, which included one sample each of the drinking water, feed, floor, litter and soil were collected.

#### **Data and sample collection**

A questionnaire was developed and used to collect information on the farm and the animals. The cloacal swabs were inoculated into buffered peptone water (BPW) while the environmental samples were placed in sterile plastic bags. These samples were transported in a chilled box at 4°C to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia Serdang for immediate bacteriological analysis.

#### **Bacteriological analysis**

The environmental samples were homogenized in BPW (1:10). The environmental and the cloacal samples were then incubated overnight at 37°C. Each overnight culture was plated onto vancomycin supplemented Slanetz-Bartley agar (SBA) (20ug/ml) (Merck) and VRE agar (Oxoid) and incubated for 24 to 48 hours at 37°C. One to two colonies that had a typical appearance of enterococci on both SBA and VRE agar were selected and purified on SBA without the vancomycin supplement. Each purified culture was then subjected to Grams stain and biochemical tests, which included sulphide indol motility (SIM; Oxoid), bile esculin agar (BEA; Oxoid) and catalase test. Those showing typical biochemical characteristic of *Enterococcus* were then speciated using the API STREP 20 (BioMerieux<sup>®</sup>, Marcy l'Etoile, France) test kit.

#### **Statistical analysis**

All data were managed and analysed using SPSS ver. 12. The prevalence data from each visit was transformed into arcsine form to enable repeated ANOVA to be performed.

## **Results**

#### **Prevalence of VRE and risk factor associated with the occurrence of the organism**

Two hundred and fifty four (47%) samples were positive for VRE from a total of 540 cloacal samples cultured and 16 (17.8%) of the 90 environmental samples taken at the farms. Day-old chick samples in five of six broiler farms were found positive for VRE. The farm prevalence of VRE in DOC (Visit 1) ranged from: 0% to 33% (mean = 18.2;sd = 13.06); in 21-day-old chickens (Visit 2) between 20% to 100% (mean = 67.77; sd = 38.87); and in 40-day-old (Visit 3) between 3.3% to 88% (mean = 54.98; sd = 32.31). The prevalence of VRE generally increased on Visit 2 sampling and slightly declined on Visit 3 sampling. *Enterococcus durans* (40.6 %) was the predominant species isolated followed by *E. faecalis* (33%), *E. faecium* (23.2%), *E. gallinarum* (1.8%) and *E. avium* (1.4%). There was a significant difference between the prevalence of VRE between visits ( $F= 5.83$ ;  $p = 0.027$ ). Bonferroni pairwise comparison revealed that as the chicken matured (from DOC to 20 days old) the prevalence of VRE increased significantly ( $p = 0.006$ ). The prevalence then declined slightly (from 20-day olds to 40-day olds), however the decline in prevalence was not significant ( $p = 0.122$ ). The type of housing system which was directly correlated with the age of the farm was found to affect the prevalence of the organism significantly ( $F = 15.45$ ;  $p = 0.017$ ). A significant interaction was obtained between visit and type of housing system to the prevalence of VRE ( $F = 7.06$ ;  $p = 0.017$ ). Farms with open-house systems showed a higher prevalence of VRE when the birds were 20 days and 40 days old. There was not much difference in the prevalence of VRE in the close-house systems between visits.

## **Discussion**

More than 40% of the samples from the broiler chickens were positive for VRE. This finding was comparable with that of Seong *et al* (2004) who found 41.6% of broiler faecal samples contained VRE, but the finding was lower compared to the study on broilers and turkeys by Borgen *et al* (2000) who found VRE in 80% of the samples. Studies carried out in Malaysia on chicken carcasses from the markets recorded lower occurrence rates (21.8% and 2%) of VRE (Ong *et al*, 2002; Radu *et al*, 2001). However, the methodology used and the processing of the bird carcasses could have played a role in the differences observed. In this study, VRE was isolated from DOCs. In addition, the prevalence of VRE increases significantly as the chickens matured (ie from DOC to 21-day-old). The sampled populations were closed populations and as such, highly suggest a horizontal transmission of infection or colonisation of VRE from one bird to another within the flock, mainly through contamination and recontamination of materials and environment. Isolation of VRE from the environmental samples, even at the beginning of a new poultry batch after the farm has been cleaned and disinfected, is in agreement with the finding of Borgen *et al* (2000). Enterococci are known to be exceedingly hardy and are able to tolerate a wide variety of growth conditions (Huycke *et al*, 1998). The slight decline in the prevalence of VRE in 40-day-old compared to 21-day-old birds could be indicative of the transitory nature of the bacterial resistance to the antibiotic. However, this supposition needs further investigation. In Europe, several studies have linked the supplementation of avoparcin in animal feed with cross-resistance to the drug vancomycin. As a result, high prevalence of VRE in animals was observed (Bager *et al*, 1997, 1999; Wegener 1999). It is unclear if avoparcin is used by the Malaysian feed millers because the ingredients added to the chicken feed were not specified. However, in the Food Regulation Act of 1985, the list of maximum residue limits for veterinary drugs includes avoparcin. It is recommended that Malaysia ban the use of avoparcin in animal husbandry and that avoparcin be removed from the list mentioned above.

In conclusion, the data analysed showed that: (i) the prevalence of VRE was very high in the sampled broiler populations and higher prevalence was observed in the close-house farms; (ii) the prevalence increased as the chickens matured; (iii) VRE was isolated from DOC which may indicate the possibility of the hatcheries as sources of perpetuation.

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