

THE DETECTION AND COMPARISON OF ANTIMICROBIAL RESISTANCE PATTERN OF VANCOMYCIN-RESISTANT ENTEROCOCCI AND *SALMONELLA* ISOLATED FROM EGGS OF COMMERCIAL LAYERS AND FREE-RANGE CHICKENS

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SUMMARY

The prevalence of vancomycin-resistant enterococci (VRE) and *Salmonella* in eggs produced by free-range chickens and commercial layers was compared to establish the microbial safety of the food and to determine if management, especially feeding management and the environment, play a role in the occurrence of the two organisms. The isolates were subjected to an antibiotic sensitivity test to determine the antibiotic resistance pattern. It was observed that there was a slight difference in the occurrence of the organisms and in the pattern of antibiotic resistance of isolates from free-range chickens' eggs and those from commercial layers. *Enterococcus* was detected in 10% and 7.7% of eggs from commercial layers and free-range chickens respectively. *Salmonella* spp occurred in 7.5% and 12.8% of commercial layers and free-range chicken eggs, respectively. The study suggests that free-range eggs may not be as wholesome as often believed and that the environment plays an important role in the development of antibiotic resistance.

Keywords: *Salmonella*, vancomycin-resistant enterococci, antibiotic resistance, eggs

INTRODUCTION

The development of antibiotic resistance in important human pathogens is a critical public health issue. In recent years, the use of antibiotics in food animals has drawn the attention of researchers and the public due to its affiliation with the antibiotic resistance development of human pathogens. Although the resistance to antibiotics is contributed to largely by human medical activities (i.e. antibiotic therapy) (WHO, 1997), there is a general consensus among scientists that feeding food animals with similar drugs has led to the development of antibiotic resistance or cross-resistance when humans, in turn, consume the meat or its product (Klare *et al.*, 1995; Bates, 1997). The off-label, sub-therapeutic use of antibiotics as a preventative measure, or as a feed-additive to improve weight gain is widely believed to have accelerated antibiotic resistance development (Jensen, 1998; Aarestrup and Wegener, 1999).

Salmonella and vancomycin-resistant enterococci (VRE) are two human pathogens that in recent years have been identified as strains that can resist multiple antimicrobials (Lathers, 2001). *Salmonella* is a classic organism known to cause important diseases in both animals and humans. On the other hand, VRE are those enterococci that are resistant to vancomycin. This group of enterococci is not known to cause diseases in animals but is gaining recognition as an agent causing nosocomial infections in man (Bates, 1997; Lathers 2001). There are several species within the *Enterococcus* genus and among those, only *E. faecium* and *E. faecalis* have been

documented to cause diseases in humans (CDC, 1993). This paper describes the detection of VRE and *Salmonella* in commercial layer and free-range or 'village' chicken eggs and the comparison of antibiotic resistance patterns. The role of diet and environment in the development of antibiotic resistance is also discussed.

MATERIALS AND METHODS

Free-range 'village' chicken eggs were obtained from the locals who maintained a backyard chicken shed. The eggs from the village chickens and from commercial layers were brought to the Veterinary Public Health Laboratory of the Faculty of Veterinary Medicine, University Putra Malaysia in their natural state and immediately processed.

Sampling the egg shell

A sterile cotton swab moistened with buffered peptone water (BPW) was used to swab the whole eggshell surface. The swab was inoculated into 10 ml of BPW and incubated at 35°C for 18-24 hours.

Sampling the egg content

The eggs were washed under running water and scrubbed with detergent. Then the eggs were immersed in 2% sodium hypochloride solution for 10-15 min. Each egg was cracked using a sterile forceps and the content was drained into a sterile plastic bag. The white and yolk

Table 1: Occurrences of *Enterococcus* species and *Salmonella* spp. on egg shell and homogenized egg content of commercial layers and free-range chickens

Organism	Commercial layer eggs		Free-range chicken eggs	
	Egg shell	Homogenized egg content	No Positive (%)	
			Egg shell	Homogenized egg content
<i>E. faecium</i>	0	2 (5)	3 (7.7)	0
<i>E. gallinarum</i>	2 (5)	0	0	0
<i>Salmonella</i> spp	0	3 (7.5)	1 (2.6)	4 (10.2)

were thoroughly homogenized. One ml of the mixture was inoculated into 9 ml of BPW.

VRE

The overnight culture was plated onto vancomycin supplemented Slanet-Bartley agar (SBA) (20 µg/ml) (Merck) and VRE agar (Oxoid) and incubated for 24-48 h at 35°C. One to two colonies that showed typical appearance of enterococci on both SBA and VRE agar were selected and purified on SBA without the vancomycin supplement. The purified culture was then subjected to biochemical tests, which included sulphide indol motility (SIM; Oxoid), bile esculin agar (BEA; Oxoid) and catalase test. Those showing typical biochemical characteristic as genus *Enterococcus* were tested using the API STREP 20 (BioMerieux®, Marcy l'Etoile, France) test kit.

Salmonella

0.1 ml of the overnight culture in BPW was inoculated into Rappaport-Vassiliadis (RV; Merck). The RV was then incubated at 42°C for 18-24 h and a loopful was plated onto XLT-4 agar (Merck). Suspect colonies were then tested using biochemical tests which include triple sugar iron (TSI; Oxoid), lysine iron agar (LIA; Merck), and urease test (Merck). Genus determination was performed using polyvalent O and H antisera (MDV Diagnostic, Australia).

Antibiotic sensitivity testing

The antibiotic sensitivity pattern positively identified *Salmonella* or *Enterococcus* was determined by the disc diffusion method using Mueller-Hinton agar (Merck). The antibiotics tested are listed in Table 2 and Table 3. Interpretive criteria were published by the National Committee for Clinical Laboratory Standards (NCCLS).

RESULTS

Thirty-nine free-range eggs and 40 commercial eggs were sampled for the study. The free-range eggs were obtained from three separate areas and owners. The commercial eggs purchased from retail outlets were from three different commercial layer farms. The commercial eggs were labelled 'Antibiotic Free'.

Four isolates from commercial eggs and three isolates from free-range eggs were identified as *Enterococcus* species. The prevalence of the bacteria in commercial eggs and free-range eggs was 10% and 7.7%, respectively. *Salmonella* sp. was isolated from eight samples; three isolates from commercial layers eggs (7.5%) and five from free-range chickens' eggs (12.8%) (Table 1).

Antibiotic resistance pattern - Commercial layer eggs

Tables 2 and 3 show the antibiotic resistance for each isolate.

E. faecium isolates — the resistance rate for ampicillin, compound sulphonamide, erythromycin and vancomycin was 0%. Resistance rate was 100% for cephadrine, cephalothin, nalidixic acid, kanamycin and streptomycin.

E. gallinarum isolates — the resistance rate for ampicillin, kanamycin, and vancomycin was 0%. Resistance rate was 100% for compound sulphonamide, cephadrine and cephalothin.

Salmonella isolates — the resistance rate for enrofloxacin, ciprofloxacin, streptomycin and erythromycin was 0%. Resistance rate was 100% for ampicillin and compound sulphonamide.

Antibiotic Resistance pattern - Free-range eggs

E. faecium isolates — the resistance rate for ampicillin, enrofloxacin, ciprofloxacin and erythromycin was 0%. Resistance rate was 100% for compound sulphonamide.

Table 2: Antibiotic resistance pattern of *E. faecium* and *E. gallinarum* isolates from commercial and free range eggs to 11 antimicrobials

Antimicrobial	Conc(µg)	Species*	Source	Resistant isolates(%)
Ampicillin	10	EF	CL	0
			FR	0
		EG	CL	0
Sulphonamide	300	EF	CL	0
			FR	3 (100)
		EG	CL	2 (100)
Enrofloxacin	5	EF	CL	1 (50)
			FR	0
		EG	CL	1 (50)
Cephadrine	30	EF	CL	2 (100)
			FR	1 (33)
		EG	CL	2 (100)
Cephalothin	30	EF	CL	2 (100)
			FR	2 (67)
		EG	CL	2 (100)
Ciprofloxacin	5	EF	CL	1 (50)
			FR	0
		EG	CL	1 (50)
Nalidixic Acid	30	EF	CL	2 (100)
			FR	0
		EG	CL	1
Kanamycin	30	EF	CL	2 (100)
			FR	1 (33)
		EG	CL	0
Streptomycin	10	EF	CL	2 (100)
			FR	2 (67)
		EG	CL	1 (50)
Erythromycin	15	EF	CL	0
			FR	0
		EG	CL	1 (50)
Vancomycin	30	EF	CL	0
			FR	1 (33)
		EG	CL	0

CL: commercial layer; FR: free-range

EF: *Enterococcus faecium*; EG: *Enterococcus gallinarum*

* EG was not isolated from any free-range eggs

Salmonella isolates — the resistance rate for enrofloxacin and ciprofloxacin was 0%. Resistance rate was more than 75% for ampicillin, compound sulphonamide, nalidixic acid, streptomycin and erythromycin.

DISCUSSION

In Malaysia, free-range or 'village' chickens are commonly raised by the locals. The free-range chickens are defined as native chickens that are let loose and free to roam out-of-door at-will and rummage for food only to return to the shed at dusk; some roost on trees or seek shelter below elevated houses (Saleha, 1997). The chickens sometimes receive household scraps, and are presumably not fed with any type of commercial feed or supplements and not treated with any type of medications.

It is common knowledge that free-range chickens usually have a longer life span than commercial layer chickens. This can be attributed to the fact that village chickens are raised not for commercial reasons, but usually as a hobby or for family consumption. They are very poor and slow weight-gainers, however, free-range eggs are popular because people believe that the eggs produced are more wholesome than the commercial eggs. In the US, free-range eggs are gaining popularity due to their completely organic association and as being drug and pesticide free. In Malaysia, the eggs are used for medicinal purposes, often eaten raw because people believe that the eggs are more nutritious and safer than the commercial layer eggs.

According to Giraffa (2002), *Enterococcus* can be found in soils, on plants and in water. Therefore, the presence of *Enterococcus* on free-range eggs was not surprising because free-range chickens often lay eggs in

Table 3: Antibiotic resistance pattern of *Salmonella* isolates from commercial and free-range eggs to 9 antimicrobials

Antimicrobial	Conc (µg)	Source	Resistant isolates (%)
Ampicillin	10	CL	2 (100)
		FR	3 (75)
Sulphonamide	300	CL	2 (100)
		FR	3 (75)
Enrofloxacin	5	CL	0
		FR	0
Cephadrine	30	CL	1 (50)
		FR	1 (25)
Cephalothin	30	CL	1 (50)
		FR	1 (25)
Ciprofloxacin	5	CL	0
		FR	0
Nalidixic Acid	30	CL	1 (50)
		FR	4 (100)
Streptomycin	10	CL	0
		FR	4 (100)
Erythromycin	15	CL	0
		FR	4 (100)

CL: commercial layer

FR: Free-range

bushes or in nests made from dried grass.

Ampicillin and aminoglycosides are the drugs of choice for the treatment of enterococcal infection (Calia, 1996). The resistance to the two drugs was documented to have generally increased over the years (Herman and Gerding, 1991; Grayson *et al.*, 1991; Boyce *et al.*, 1992). In this study, all *E. faecium* isolates were susceptible to ampicillin; however, more than 60% were resistant to the aminoglycosides antibiotic group.

The prevalence of *Salmonella* in free-range eggs was higher than in the commercial layer eggs. As *Salmonella* is one of the more important pathogens in the poultry industry, trans-ovarian transmission of the pathogen to the eggs has been markedly reduced through testing and culling of affected layers (Courtts, 1981). Therefore, prevalence of *Salmonella* in the commercial layer environment was expected to be lower.

The present study found that *E. faecium* isolates from commercial chickens eggs were resistant to multiple antibiotics tested. Even though labelled 'antibiotic-free', these eggs still harbour multiple resistant *E. faecium* as they come from an environment where antibiotics are frequently used. However, our findings revealed the contrary for *Salmonella* isolates, whereby more *Salmonella* isolates from free-range eggs were observed to be multiple-antibiotic resistant, even though village chickens were from an environment where antibiotics are not used. This phenomenon may be explained by the observation by Mamber and Katz (1985). In their study, they concluded that multiple antibiotic resistances could occur in almost any enteric bacilli, even if the animals were not given antibiotic supplements or medication, as long as they were exposed to an environment where other

antimicrobial resistant strains were present. Therefore, as village chickens generally have a longer life span than most commercial layers, it is possible that antibiotic resistance was acquired over time through genetic material transfer from other resistant organisms. In addition, the resistance could have also been acquired from prolonged exposure to food with antibiotic residues, e.g. from leftovers of human food tainted with antibiotics.

CONCLUSIONS

Although there were some differences between the antibiotic resistance pattern of VRE and *Salmonella* isolates from the village chicken eggs and commercial eggs, these differences were not remarkable. However, the findings suggest that the assumptions about the free-range eggs as safer, and more wholesome than commercial eggs may not be true. Further studies with larger samples will determine if the differences observed in this study are of biological importance or due to chance occurrence.

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