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ORIGINAL ARTICLE

DETECTION OF VANCOMYCIN-RESISTANT *ENTEROCOCCUS* SPP. (VRE) FROM POULTRY

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Twenty-eight isolates of *E. faecalis* and 5 isolates of *E. hirae* were isolated from chicken samples obtained from markets in Sri Serdang, Selangor. They were tested for susceptibility to vancomycin and other antimicrobial agents. All of the isolates showed multiple resistance to the antibiotic tested. All *Enterococcus* spp. were resistant (100%) to ceftaxidime, cephalothin, erythromycin, gentamicin, kanamycin, nalidixic acid and streptomycin. Resistance was also observed to norfloxacin (97%), tetracycline (91%), penicillin (85%), bacitracin (82%), chloramphenicol (61%) and the least resistance was to ampicillin (27%). High prevalence to vancomycin resistance was detected among the *E. faecalis* (27of 28) and *E. hirae* (4 of 5) isolates. The multiple antibiotic resistance index ranging between 0.64 to 1.0 showed that all strains tested originated from high-risk contamination. Plasmid profile analysis of *Enterococcus* spp. revealed plasmid DNA bands ranging in size from 1.3 to 35.8 megadalton but some isolates were plasmidless. No correlation could be made between plasmid patterns and antibiotic resistance.

Key words : *Enterococcus spp., vancomycin-resistant, plasmid, poultry*

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Introduction

Enterococcus species usually inhabit the intestines of human and other animals. These organisms were considered as a part of normal flora of the bowel, genital tract with some also being found on the skin, vaginal secretions and in the perineal area. The genus *Enterococcus* are gram-positive cocci that are catalase negative, occur singly, in pair and short chains.

Enterococci have been increasingly involved in nosocomial infections, sometimes as a cause of hospital outbreaks (1). In recent years, they have emerged as pathogens in a growing number of serious nosocomial and urinary tract infections including bacteremia and intraabdominal (2,3). The isolation of strains resistant to many antibiotic therapies has become an important public health concern (4,5,6). Once, the glycopeptide antibiotic agent, vancomycin was useful in the treatment of severe infections due to gram-positive bacteria (7,8). Unfortunately, resistance to vancomycin had been reported (9).

Our aim was to isolate and investigate the resistance of enterococcal poultry isolates to various antimicrobial agents including glycopeptide (vancomycin) as well as to determine their plasmid profiles.

Materials and Methods

Isolation of Vancomycin-Resistant Enterococci (VRE)

All thirty-three isolates of Enterococci were isolated from chicken meat samples (chicken breasts, chicken legs and other chicken parts) obtained from markets in Sri Serdang, Selangor.

Approximately 25 g of each poultry product was rinsed in 225 ml of azide dextrose broth and homogenized with a stomacher for 1 min. After overnight incubation at 37^{0}C, 0.1 ml of the diluted sample was plated on Slanetz and Bartley agar (SBA) supplemented with 20 μg/ml of vancomycin. The agar plates were incubated aerobically at 37^{0}C for 24 hour. Typical red colonies from each SBA plate were randomly isolated and investigated further.

Species Identification

Presumptive identifications of the *Enterococcus* spp. were performed by using the following characteristics: Gram stained reaction, colony morphology, growth and blackening of bile-esculin agar, growth in the presence of 6.5% NaCl and growth at 10^{0}C and 45^{0}C, the presence or absence of catalase and acidification of glucose with the production of gas (10).

Identification of the strains was further investigated to the genus level by growth and biochemical reactions as described by Facklam and Collins (11).

Plasmid isolation

The plasmid DNA of *Enterococcus* spp. strains were screened by the alkaline lysis method of Birnboim and Doly (12) with slight modification. The products were then electrophoresed for 1 hour at 150V on a 0.8% agarose gel. After staining the gel with ethidium bromide (0.5 μg/ml), the photograph was taken. Molecular mass of the plasmid was determined by approximate comparison with plasmid of known molecular weight, *E.coli* V517 that harboured 8 plasmid of 1.4 to 35.8 MDa (13).

Antimicrobial Susceptibility Testing

Table 1: *Frequency of antibiotic resistance of the thirty-three* Enterococcus *spp. tested*

	No. (%) of resistant strains		
Antibiotic	**E. faecalis (28 strains)**	**E. hirae (5 strains)**	**Total (33 strains)**
Ampicillin	7 (25)	2 (40)	9 (27)
Bacitracin	25 (89)	2 (40)	27 (82)
Chloramphenicol	18 (64)	2 (40)	20 (61)
Ceftazidime	28 (100)	5 (100)	33 (100)
Cephalothin	24 (86)	5 (100)	33 (100)
Erythromycin	28 (100)	5 (100)	33 (100)
Gentamicin	27 (96)	5 (100)	32 (97)
Kanamycin	28 (100)	5 (100)	33 (100)
Nalidixic acid	28 (100)	5 (100)	33 (100)
Norfloxacin	27 (96)	5 (100)	32 (97)
Penicillin	24 (86)	4 (80)	28 (85)
Streptomycin	28 (100)	5 (100)	33 (100)
Tetracycline	25 (50)	5 (100)	30 (91)
Vancomycin	27 (96)	4 (80)	31 (94)

***Table II**: Plasmid profiles and antibiotic resistance patterns of* Enterococcus *spp. isolates*

Strains	Antibiotic resistance[ab]	MAR Index	Plasmid profiles (MDa)[c]
HTF101	BCCazCfEGmKNaNorPSTeVa (1)	0.93	35.8 (1)
HTF102	AmBCCazCfEGmKNaNorPSTeVa (2)	1.0	35.8, 2.9 (2)
HTF103	BCCazCfEGmKNaNorPSTeVa (1)	0.93	35.8, 1.9, 1.3 (3)
HTF104	AmBCCazCfEGmKNaNorPSTeVa (2)	1.0	35.8, 1.9, 1.3 (3)
HTF105	BCCazCfEGmKNaNorPSTeVa (1)	0.93	3.8 (4)
HTF106	BCCazCfEGmKNaNorPSTeVa (1)	0.93	35.8 (1)
HTF107	BCCazCfEGmKNaNorPSTeVa (1)	0.93	-[d]
HTF108	BCCazCfEGmKNaNorPSTeVa (1)	0.93	-
HTF109	BCazCfEGmKNaNorPSTeVa (3)	0.86	35.8, 5.8 (5)
HTF110	AmBCCazCfEGmKNaNorPSTeVa (2)	1.0	5.0 (6)
HTF111	AmBCCazCfEGmKNaNorPSTeVa (2)	1.0	1.9, 1.3 (7)
HTF112	AmBCCazCfEGmKNaNorPSTeVa (2)	1.0	35.8, 1.9, 1.3 (3)
HTF113	AmBCazEGmKNaNorPSTeVa (4)	0.86	-
HTF114	BCCazCfEGmKNaNorPSTe (5)	0.86	35.8 (1)
HTF115	CCazEGmKNaNorSTeVa (6)	0.71	-
HTF116	CCazCfEGmKNaNorPSTeVa (7)	0.86	-
HTF117	AmBCCazCfEGmKNaNorPSTeVa (2)	1.0	-
HTF118	BCCazCfEGmKNaNorPSTeVa(1)	0.93	-
HTF119	BCCazCfEGmKNaNorPSTeVa(1)	0.93	-
HTF120	BCazEGmKNaNorPSTeVa(8)	0.79	-
HTF121	BCazCfEKNaNorPSVa(9)	0.71	-
HTF122	BCazCfEGmKNaNorPSTeVa(3)	0.86	-
HTF123	BCCazCfEGmKNaNorPSTeVa(1)	0.93	-
HTF124	BCazCfEGmKNaSTeVa(9)	0.71	-
HTF125	BCazCfEGmKNaNorSTeVa(11)	0.79	-
HTF126	BCazEGmKNaNorPSTeVa(8)	0.79	-
HTF127	CazCfEGmKNaNorPSVa (12)	0.71	-
HTF128	BCazCfEGmKNaNorSTeVa (11)	0.79	-
HTH101	CazCfEGmKNaNorSTe (13)	0.64	-
HTH102	AmCazCfEGmKNaNorPSTeVa (14)	0.86	6.0, 3.7 (8)
HTH103	AmBCazCfEGmKNaNorPSTeVa (15)	0.93	7.0, 3.7, 2.8 (9)
HTH104	BCCazCfEGmKNaNorPSTeVa (1)	0.93	7.0, 3.7, 2.8 (9)
HTH105	CCazCfEGmKNaNorPSTeVa (7)	0.86	1.8, 1.5 (10)

[a]Tested for ampicillin (Am), bacitracin (B), chloramphenicol (C), ceftazidime (Caz), cephalothin (Cf) erythromycin (E), gentamicin (Gm), kanamycin (K), nalidixic acid (Na), norfloxacin (Nor), penicillin (P), streptomycin (S), tetracycline (Te), vancomycin (Va)

[b,c] Number in parenthesis indicates antibiotypes group and plasmid patterns group

[d]None detected

All isolates identified as enterococci were tested by disk diffusion tests on tryptic soy agar (11). All strains were tested for their susceptibility to ampicillin at 10 μg, bacitracin at 10 μg, chloramphenicol at 30 μg, ceftazidime at 30 μg, cephalothin at 30 μg, erythromycin at 15 μg, gentamicin at 10 μg, kanamycin at 30 μg, nalidixic acid at 30 μg, norfloxacin at 30 μg, penicillin at 10 U, streptomycin at 10 μg, tetracycline at 30 μg and vancomycin at 30 μg.

The multiple antibiotic resistance (MAR) index of isolates was defined as a/b where 'a' was the number of antibiotics to which the isolate was resistance and 'b' was the total number of antibiotics tested (14).

Results

From the 33 vancomycin-resistant Enterococci (VRE) isolated from the chicken meat examined, 28 (85%) strains were identified as *E. faecalis* and 5 (15%) as *E. hirae*. Generally the characteristics of the isolates agreed with previous studies that the genus *Enterococcus* comprised of gram-positive cocci that are catalase negative, grow in 6.5% NaCl and at pH 9.6. They grow both at 10^0C and 45^0C and none produced gas from glucose. They also grew on and blackened 40% bile-esculin agar (11, 15, 16). All thirty-three isolates of Enterococci were resistant to 9 or more antibiotic tested. The highest prevalence of resistance observed among the isolates were against ceftazidime, cephalothin, erythromycin, gentamicin, kanamycin, nalidixic acid and streptomycin (100%). The least resistance was observed for ampicillin (27%). Table I showed the resistant pattern among the *Enterococcus* spp. tested. The results of plasmid profile analysis among the *Enterococcus* spp. isolates were shown in Table II. Fifteen of the isolates harbour one or more plasmid DNA bands ranging in sizes from 1.3 to 35.8 megadalton.

Discussion

The isolation of vancomycin-resistant enterococci (VRE) species in this study was to investigate the importance of chicken meat as a possible source for the transfer of VRE. The results obtained showed the presence of VRE in the chicken samples examined and similar reports have been published on the occurrence of *Enterococcus* spp. from animal sources (17, 18, 19, 20, 21).

There is little information on the resistance to antibiotics among *Enterococcus* spp. in Malaysia. In this study, 94% of the isolates were vancomycin-resistant and more than 50% of the Enterococci isolates acquired high-level resistance to other antibiotics tested, reflecting the distribution of aminoglycoside resistance worldwide and resistance to other antibiotics among VRE worldwide (22). The resistance patterns in this study generally agreed with the observations reported by Murray (1) and Son *et al*. (23) on the prevalence of multiple drug-resistant enterococci. Twenty-seven of 28 *E. faecalis* and 4 of 5 *E. hirae* were resistant to vancomycin, which indicated the high prevalence of vancomycin-resistant enterococci from chicken samples tested in the study area. In addition, all isolates of *Enterococcus* spp. from poultry sources used in this study had multiple antibiotic resistance (MAR) indices of 0.64 to 1.0, indicating that all strains originated from high-risk sources (14). Elsewhere, a frequent occurrence of antimicrobial resistance enterococci has been observed among food animals and food of animal origin (17, 19, 21, 24, 25). Taken together, the results of this study and those cited above suggested that food animals might be a reservoir of resistant enterococci and resistance gene capable of transferring to human through the food chain.

The results of the plasmid screening generally agreed with previous studies by Son *et al*. (23) who reported the occurrence of small and large plasmid DNA compared to Boyce *et al*. (26) who revealed that all isolates of VRE examined contained a common 40 MDa plasmid. Taken together, these results suggest that plasmids in VRE are of variable size. Bacterial plasmid are known to confer a variety of phenotypic modifications and genetic flexibility upon their host by carrying genes that may code for toxin production and antibiotic resistance (27). Plasmid screening by agarose gel electrophoresis revealed 10 plasmid profiles scattered in 33 of the isolates. Fifteen antibiotypes were identified among 33 *Enterococcus* spp. strains based on the evidence of resistance patterns. Though vancomycin resistant among clinically important Gram-positive species had not been widely reported before 1986, over the last decade has witness the emergence of glycopeptide resistance from negligible rate to clinically problematic levels (28, 29, 30). Glycopeptide resistance in enterococci is thought to be principally plasmid-mediated and the ability to transfer resistant genetic material among Gram-posiotive strains and species has been demonstrated (29, 30, 31, 32). This renders *Enterococcus* species

that have been previously considered of minor clinical importance, significant if associated with either multiple resistance factors or as a reservoir of resistance genes as observed in this study. However, at this stage of this study no specific correlation between the antibiotic patterns and plasmid profiles was observed. Further evidence on the correlation of the presence of plasmids and antibiotic resistance could be obtained by conjugation, transformation or curing experiments.

In conclusion, our findings showed that multiple resistant VRE isolates are already present in poultry and thus, there is every reason to be concerned as human infection due to VRE may stem from poultry sources.

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