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Research article

Antimicrobial and heavy metal resistance in commensal enterococci isolated from pigs

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ABSTRACT

Antibiotic resistance in animal isolates of enterococci is of public health concern because of the risk of transfer of antibiotic resistance isolates or resistance determinants to consumers via the food chain. In this study, phenotypic and genotypic resistance in 192 pig isolates of enterococci to ampicillin, avilamycin, avoparcin, bacitracin, flavophospholipol, gentamicin, narasin, tetracycline, tiamulin, tylosin, vancomycin, virginiamycin, copper and zinc were investigated by susceptibility test and molecular methods. Resistance rates varied between the species but all isolates were susceptible to ampicillin, avilamycin, avoparcin, gentamicin and narasin but resistant to tetracycline and tylosin and intermediately resistant to copper. Only *Enterococcus gallinarum* and *Enterococcus casseliflavus* were resistant to vancomycin and virginiamycin resistance was present in less than half the *Enterococcus faecium* isolates. Zinc resistance was largely confined to *Enterococcus faecalis* but bacitracin resistance was uncommon in *E. faecalis* in comparison with the other species. Tiamulin resistance was common in all species except *E. casseliflavus*. Resistance to flavophospholipol was detected in most *E. faecium* isolates and in a high proportion of *E. gallinarum*, *E. casseliflavus* and *E. hirae/durans* but was only found in one isolate of *E. faecalis*. No *tet*O, *rpl*C, *rpl*D, *van*A, *van*B, *vat*A and *vat*D genes were found. The presence of *erm*B, *tet*L, *tet*M, *tcr*B, *aac*6-*aph*2, *tet*K, *tet*S, *van*C1, *van*C2, *lsa*A, *lsa*B and *vat*E varied between the species and largely corresponded to the susceptibility phenotype. The findings show that resistance to antibiotics of high clinical significance for nosocomial *Enterococcus* infections is absent, whereas antimicrobial resistance was detected for some other antibiotics including bacitracin, flavophospholipol, tetracycline, tiamulin, tylosin and virginiamycin.

1. Introduction

Antimicrobial resistance in enteric bacteria from animals is a public health concern because of transfer of resistance organisms and resistance genes to humans via the food chain. Resistance in potentially pathogenic enterococci such as *Enterococcus faecalis* and *Enterococcus faecium* isolated from animals has been well documented (Hershberger et al., 2005; Sørensen et al., 2001) but there is little published information on resistance in other species of enterococci. Antibiotics are administered to animals to promote feed efficiency and improve the rate of weight gain in livestock as well as prophylactically and therapeutically. Metals such as copper and zinc and their derivative chemical compounds also have antimicrobial activity (Antunes et al., 2003). Livestock feeds are often supplemented with copper and/or zinc salts for their growth promotion activity.

Resistance to antimicrobials, including heavy metals, is important for bacterial survival in contaminated environments. Many antibiotic and heavy metal resistance genes are located on the same mobile genetic elements (MGEs)

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such as plasmids, transposons and integrons (Aminov and Mackie, 2007). These multiple-gene MGEs are exchanged between the bacteria living in areas contaminated by heavy metals. Therefore, it can be concluded that the natural selective pressure imposed by heavy metals may indirectly select for the resistance to antibiotics.

In this project, the antimicrobial resistance profile of pig enterococci to 12 antibiotics and two heavy metals was investigated by susceptibility test and molecular methods. These antimicrobials, including ampicillin, avilamycin, avoparcin, bacitracin, flavophospholipol, gentamicin, narasin, tetracycline, tiamulin, tylosin, vancomycin, virginiamycin, copper and zinc, were chosen due to their importance in human and veterinary medicine and animal husbandry.

2. Materials and methods

2.1. Bacterial isolates

One hundred and ninety two enterococci (75 *E. faecalis*, 68 *E. faecium*, 21 *E. gallinarum*, 13 *E. casseliflavus* and 15 *E. hirae/durans* (undifferentiated)) isolated from the intestinal tracts of slaughter pigs (Barton et al., 2005) were used in this project. These isolates had been identified using biochemical tests and by PCR.

2.2. Antimicrobial susceptibility test

All isolates were tested for antimicrobial resistance by agar dilution method (CLSI, 2008). Appropriate concentrations of antibiotics and heavy metals were added to Mueller-Hinton agar to make serial dilutions of the antimicrobial agents. Copper resistance of selected isolates was tested on Mueller-Hinton agar plates containing 0, 4, 8, 12, 16, 20, 24, 28 and 32 mM copper sulfate ($CuSO_4{\cdot}5H_2O$) adjusted to pH 7 with 1 M NaOH. The resistance breakpoints for copper sulphate were considered to be $\leq$2 and $\geq$28 mM/mL (Hasman et al., 2006). The plates were then incubated at 37 °C for 16–20 h, and growth was assessed. Susceptibility testing to zinc chloride was determined on Mueller-Hinton agar plates containing two-fold serial dilutions of zinc chloride (0.25 – 16 mM) with the pH adjusted to 5.5. The resistance breakpoints for zinc chloride were considered to

Table 1
An overview of target genes and PCR primers.

Drug	Gene		Sequences (5′–3′)	G	°C	bp	Reference
AVO, VAN	*van*A	F	GGGAAAACGACAATTGC	1	47	732	Dutka-Malen et al. (1995)
		R	GTACAATGCGGCCGTTA				
GEN	*aac*6-*aph*2	F	CCAAGAGCAATAAGGGCATA	2	52	220	Rouch et al. (1987)
		R	CACTATCATAACCACTACCG				
TET	*tet*K	F	TTAGGTGAAGGGTTAGGTCC	2	52	718	Aarestrup et al. (2000)
		R	GCAAACTCATTCCAGAAGCA				
TET	*tet*L	F	CATTTGGTCTTATTGGATCG	4	47	488	Aarestrup et al. (2000)
		R	ATTACACTTCCGATTTCGG				
TET	*tet*M	F	GTTAAATAGTGTTCTTGGAG	1	47	657	Aarestrup et al. (2000)
		R	CTAAGATATGGCTCTAACAA				
TET	*tet*O	F	GATGGCATACAGGCACAGAC	2	52	614	Aarestrup et al. (2000)
		R	CAATATCACCAGAGCAGGCT				
TET	*tet*S	F	TGGAACGCCAGAGAGGTATT	3	58	660	Aarestrup et al. (2000)
		R	ACATAGACAAGCCGTTGACC				
TIA	*rpl*C	F	AACAGTTGACGCTTTAATGGGC	3	58		Gentry et al. (2007)
		R	GGCTCAATACCGAATACTGCATCGC				
TIA	*rpl*D	F	CACAGAAAACAAAGTTATCTTAGTAA	2	52		Gentry et al. (2007)
		R	GGTTTGTAATTCATGATATTAACACTT				
VAN	*van*B	F	ATGGGAAGCCGATAGTC	1	47	635	Dutka-Malen et al. (1995)
		R	GATTTCGTTCCTCGACC				
VAN	*van*C1	F	GGTATCAAGGAAACCTC	1	47	822	Dutka-Malen et al. (1995)
		R	CTTCCGCCATCATAGCT				
VAN	*van*C2	F	CTCCTACGATTCTCTTG	1	47	439	Dutka-Malen et al. (1995)
		R	CGAGCAAGACCTTTAAG				
VIR, TYL	*erm*B	F	CATTTAACGACGAAACTGGC	1, 6	45–47	738	Jensen et al. (1999)
		R	GGAACATCTGTGGTATGGCG				
VIR	*lsa*A	F	CGCTCCAGCTGTATGAGAACTGC	3	58	916–9	Dina et al. (2003)
		R	TCAAGCGATTGACTTCTTTTTTG				
VIR	*lsa*B	F	CAAGTGGCTGAATATTTGAAG	5	47	842–4	Dina et al. (2003)
		R	TCCCTTTACTTTTTCAAGATG				
VIR	*vat*A	F	TGGAGTGTGACAAGATAGGC	2	52	144–7	Soltani et al. (2000)
		R	GTGACAACAGCTTCTGCAGC				
VIR	*vat*D	F	GCTCAATAGGACCAGGTGTA	2	52	144–7	Soltani et al. (2000)
		R	TCCAGCTAACATGTATGGCG				
VIR	*vat*E	F	ACTATACCTGACGCAAATGC	2	52	904	Soltani et al. (2000)
		R	GGTTCAAATCTTGGTCCG				
Cu	*tcr*B	F	CATCACGGTAGCTTTAAGGAGATTTTC	3	58	663	Hasman et al. (2006)
		R	ATAGAGGACTCCGCCACCATTG				

G, group; °C, annealing temperature; bp, base pair; AVO, avoparcin; GEN, gentamicin; TET, tetracycline; TIA, tiamulin; TYL, tylosin; VAN, vancomycin; VIR, virginiamycin; Cu, copper.