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Macrolide resistance in porcine streptococci: a human health hazard?

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Summary: In order to obtain better insights into the possible exchange of resistance genes between human and porcine streptococci, macrolide and lincosamide resistant streptococci from tonsillar and colon swabs from pigs and pork carcass swabs were isolated and their resistance phenotypes and genotypes were determined. The sequences of the *erm*(B) genes of 21 human streptococci, 22 porcine streptococci and 15 streptococci isolated from pork carcasses were compared. From each of the 33 pigs and from 88 of 99 carcass swabs, at least one resistant streptococcal strain was isolated. The predominant phenotype was the constitutively expressed MLS_B phenotype, mostly encoded by the *erm*(B) gene. Identical *erm*(B) gene sequences were present in strains from humans, pigs and pork carcasses.

Keywords: Macrolide resistance, *erm*(B), streptococci, swine, resistance transfer

Introduction: Two types of antimicrobial drug resistance transfer from animals to humans might be of importance. The direct way of resistance transfer occurs when resistant zoonotic bacteria infect humans. The contribution of the indirect way of resistance transfer to the antimicrobial resistance problems in human medicine is less clear. Indirect way means that resistance genes from bacteria associated with animals are transferred to bacteria associated with humans. One of the most important antibiotic groups affected by resistance in humans and in pigs is the macrolide-lincosamide-streptogramin B (MLS_B) antibiotic family. The present study was carried out in order to obtain better insights in the possible exchange of resistance genes between human and porcine streptococci.

Materials and methods: Tonsillar and colon swabs were collected from 33 pigs, originating from 33 different farms in Belgium. Ninety-nine swabs were taken by swabbing the entire skin surface of pork carcasses immediately after slaughter in four slaughterhouses. The pigs originated from 35 different

Belgian farms. The swabs were inoculated on Columbia agar (Oxoid, England) supplemented with 5% sheep blood, colistin and aztreonam, supplemented with 1 µg/ml erythromycin, 8 µg/ml erythromycin or 10 µg/ml lincomycin. All colony types were purified and identified using tDNA-PCR (Baele et al., 2001). From each animal or carcass only one strain per species carrying the same resistance phenotype and genotype was included in the results. Antimicrobial susceptibility patterns of the strains were determined by disk diffusion on Columbia blood agar using the antimicrobial test tablets clindamycin, erythromycin, lincomycin and tylosin. An MLS_B phenotype isolate was defined as an isolate resistant to erythromycin, clindamycin, lincomycin and tylosin. An M phenotype isolate was an isolate resistant to erythromycin alone, and the L phenotype showed resistance to lincosamides only. The presence of the *erm*(B) and *mef*(A) genes was determined by PCR using primers derived from published sequences (Martel et al., 2001; Sutcliffe et al., 1996). The sequences of the *erm*(B) genes of 21 human streptococci (two *S. oralis-mitis* sp., eight *S. pneumoniae*, five *S. pyogenes*, one *S. salivarius*, three *S. sanguinus* and two *S. thermophilus* strains), 22 porcine streptococci (three *S. alactolyticus*, one *S. bovis*, one *S. dysgalactiae*, five *S. gallolyticus*, three *S. hyointestinalis* and nine *S. suis* strains) and 15 streptococci isolated from pork carcasses (two *S. alactolyticus*, one *S. bovis*, two *S. dysgalactiae*, two *S. hyointestinalis*, three *S. pneumoniae* and five *S. suis* strains) were determined using the BigDye Terminator Cycle Sequencing kit (PE Biosystems) and the primers 5'ATGAACAAAAATATAAAATATT3' and 5'TTATTTCCTC CCGTTAAA3'.

Results: From tonsillar and colon swabs from each of the 33 pigs and from 88 out of the 99 pork carcass swabs at least one resistant streptococcal species was isolated. Their resistance phenotypes and genotypes are presented in Tables 1 and 2. The MLS_B phenotype was most frequently detected and generally encoded by *erm*(B) genes. Sequencing of these genes from different streptococci showed a similarity between 98.7% and 100%. Identical *erm*(B) genes were present in streptococcal strains isolated from humans, pigs and pork carcasses.

Table 1. *Resistance phenotype and genotype from streptococci isolated from tonsillar and colon swabs from 33 pigs.*

	Isolated from	Number	Number MLS_B*	Number L*	Number *erm*(B) +	Number *mef*(A) +
Streptococcus alactolyticus	C	9	9		9	0
Streptococcus bovis	C	4	2	2	2	0
Streptococcus dysgalactiae	T	1	1		1	0
Streptococcus gallolyticus	C	8	8		8	0
Streptococcus hyointestinalis	C, T	4	4		4	0
Streptococcus pluranimalium	T	1	1		1	0
Streptococcus suis	C, T	33	33		32	1

C Colon swab, T Tonsillar swab, * resistance phenotype

Table 2. *Resistance phenotype and genotype from streptococci isolated from 99 pork carcasses.*

	Number	Number MLS_B*	Number M*	Number L*	Number *erm*(B) +	Number *mef*(A)+
Streptococcus agalactiae	1	1			1	0
Streptococcus alactolyticus	42	34		8	33	*1*[a]
Streptococcus bovis	7	7			7	0
Streptococcus dysgalactiae	23	15	5	2	13	8 (*1*)
Streptococcus hyointestinalis	11	11			8	*2*
Streptococcus pneumoniae	4	4			3	*1*
Streptococcus suis	43	41	2		40	3 (*1*)
Streptococcus hyovaginalis	2	1		1	1	0
Streptococcus porcinus	3	2		1	2	0

* resistance phenotype, [a] strains with MLS_B phenotype carrying only the *mef*(A) gene

Discussion: In this study, macrolide resistant streptococci were frequently found in tonsillar and colon swabs from pigs and on pork carcasses. The predominant resistance phenotype was the MLS_B phenotype. A minority of the strains showed the M-, L- and ML phenotype. A similar distribution of phenotype patterns was obtained by Lagrou et al. (2000) with Belgian *S. pneumoniae* isolates. The MLS_B phenotype was found to be encoded mainly by the *erm*(B) gene. In human streptococci in Belgium, *erm*(B) encoded resistance is also the most important mechanism in *S. pneumoniae* and *S. pyogenes* (Descheemaeker et al., 2000; Lagrou et al., 2000). Since identical *erm*(B) genes were found in porcine and in human strains, it might be possible that this gene is transferred between animal and human strains. Further studies are required to obtain better insights into possible exchange of resistance genes between human and porcine streptococcal strains. These studies should include identification of mobile DNA elements in human and porcine strains. Localisation of identical *erm*(B) genes on identical plasmids or transposons would be a further indication of transfer of these genes between these strains.

Acknowledgements: This work was supported by FOD Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu, DG4, Brussels, Belgium.

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Adaptive resistance to Biocides and implications of cross-resistance to Antimicrobial Agents in Foodborne Pathogens.

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Summary: This study was focused on the potential for adaptive resistance in *Salmonella* and *Escherichia coli* to commonly used biocides, to identify resistance strategies and any cross-resistance to antibiotics. Bacteria were serially exposed in sub-inhibitory concentrations of biocides and adaptive resistance was observed in all strains investigated. Erythromycin-resistant *Salm.* Enteritidis did not cross-resist to biocides, whereas erythromycin-resistant *Salm.* Typhimurium express cross-resistance to chlorohexidine. Benzalkonium chloride-resistant *Salm.* Virchow showed an elevated resistance to chlorohexidine, however chlorohexidine-resistant *Salm.* Virchow did not demonstrate it back. Triclosan-