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.

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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-

resistant *E. coli* O157 strains exerted decreased susceptibility to chloramphenicol, erythromycin, imipenem, tetracycline and trimethoprim and to biocides. Conversely, TLN-adapted *E. coli* O55 and K-12 did not show any cross-resistance to the antimicrobial agents tested. Possibly, domestic kitchens and places of commercial food production may provide a selective environment for bacterial adaptation, which may lead to the undesirable situation of resident strains becoming resistant and cross-resistant to other antimicrobials.

Keywords: *Salmonella* Typhimurium, *Escherichia coli* O157, Erythromycin, Chlorohexidine, Triclosan.

Introduction: Currently, pork producers are facing many challenges including food safety, pork quality and welfare standards. Antimicrobial use and resistance of pathogens associated with pork is another very important issue. There is currently much interest regarding drug administration in animals, especially with the link to the emergence of multiple antimicrobial resistant zoonotic bacterial pathogens. Research on the antimicrobial resistance of foodborne pathogens is becoming increasingly critical due to increased awareness and public concern over bacterial food safety. Thus, this study was focused on the mechanisms underlying antimicrobial resistance in *Salmonella* and *E. coli*. This may help in the future design of new antimicrobial preparations, which will help to overcome existing issues.

Materials and Methods: Antimicrobial agents and Biocides. Antimicrobial agent disks included amoxycillin (AMX), amoxycillin/clavulanic acid (AMC), chloramphenicol (C), ciprofloxacin (CIP), clindamycin (CLI), colistin sulfate (CS), fusidic acid (FD), gentamycin (GEN), imipenem (IPM), rifampicin (RIF), tetracycline (TET), trimethoprim (TMP), vancomycin (VAN), and erythromycin (ERY). Biocides included benzalkonium chloride (BKC), chlorohexidine (CHX) and triclosan (TLN).
Bacterial Identification: Random Amplification of Polymorphic DNA was employed to confirm strain continuity (Hopkins and Hilton, 2001).
Minimum Inhibitory Concentration (MIC) and Serial Passage: The MIC was determined using a standard broth dilution method carried out using a two-fold dilution of each antibacterial agent (Loughlin et al., 2002). Bacterial adaptation was performed following the technique described by Joynson et al., 2002.
Cross-resistance to Antimicrobial Agents & Biocides: Cross-resistance towards various antibiotics and biocides was determined using the Stokes' method (Anon. 1991).

Results: Bacterial Identification: Following each passage, pre-and post-adapted strains were characterised by RAPD profiling to ensure strain continuity (Fig.1). All strains shared the same RAPD profile.
MICs: The gradual passage of *Salmonella enterica* and *Escherichia coli* strains to sub-inhibitory concentrations of ERY, BKC, CHX and TLN, produced cultures capable of growth at high concentrations (Table 1). Those cultures capable to grow at the maximum concentrations of the antibacterials tested were used for the performance of the cross-resistance experiments.
Cross-resistance to Antimicrobial Agents & Biocides: The increase in the MICs through adaptation to antimicrobials tested, did confer cross-resistance in some instances. Triclosan-adapted *Escherichia coli* O157 expressed decreased susceptibility to a number of antibiotics, including chloramphenicol, erythromycin, imipenem, tetracycline, trimethoprim and to various biocides (Fig. 2), whereas other *E. coli* strains did not. *Salmonella enterica* strains did not generally show decreased susceptibility to antibiotics/biocides with the exception of *Salm.* Typhimurium and *Salm.* Virchow, which did in some instances. Generally though, in TLN-adapted *Salmonella enterica* cross-resistance between antibiotics and biocides did not occur frequently.

Figure 1: *RAPD from TLN-adapted K-12 strains. Lanes 1 to 4 represent no. of passages. M is a 1 kbp molecular weight marker.*

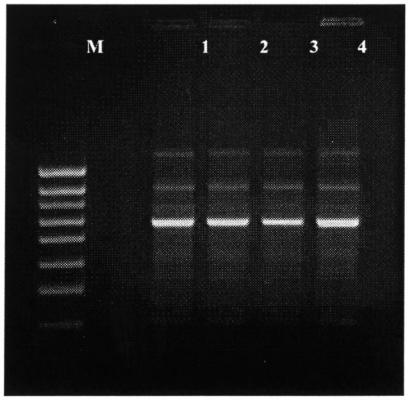
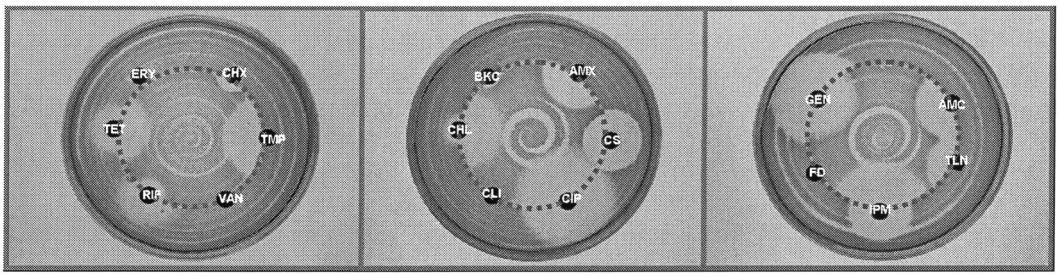


Figure 2: *Cross-resistance between TLN pre- & post-adapted E. coli O157 strains.*



Strains	ERY MIC(mg/L)	BKC MIC(mg/L)	CHX MIC(mg/L)	TLN MIC(mg/L)
Salm. **Enteritidis Parent /** Adapted	256 / 2048	32 / 256	-	16 / 512
Salm. **Typhimurium Parent /** Adapted	256 / 512	32 / 64	-	8 / 512
Salm. **Virchow Parent /** Adapted	32 / 512	4 / 256	8 / 128	16 / 1024
E. coli **O157 Parent /** Adapted	256 / 1024	16 / 1024	4 / 512	0.25 / 2048
E. coli **O55 Parent /** Adapted	-	-	-	1 / 1024
E. coli **K-12 Parent /** Adapted	-	-	-	0.125 / 1024

Table 1: *Summary of Susceptibility of pre-& post-adapted Salmonella enterica and E. coli strains to antimicrobials.*

Discussion: Cross-resistance to different antibacterial agents including quinolones and nalidixic acid, chloramphenicol, trimethoprim and in some cases b-lactam antibiotics is a common phenomenon in Gram-negative bacteria (Gutmann et al., 1995). In this study adaptive resistance in *Salmonella enterica* and *E. coli* O157, O55 and K-12 was readily achieved by passage in sublethal concentrations of antibacterial agents, which conferred cross-resistance to other antibiotics and biocides. Interestingly, adaptive resistance to TLN by *E. coli* O157 appeared to confer a marked increased sensitivity to AMC, AMX and IPM and to a lesser degree to CHL, CS and GEN. Differences between the adaptive- and cross-resistance profiles between K-12, O55 and O157 suggest that strain-specific rather than global mechanisms are underlying the resistance observed, some of which may be facilitated by the additional genes O157 is known to possess over K-12 and potentally O55 (Perna et al., 2001). No obvious correlation could be drawn between *Salmonella* serotype and resistance to a particular class of antibiotics or group of biocides, however, this does not detract from the finding that in particular strain / antibiotic / biocide combination strong evidence of cross-resistance was observed.

Conclusions: These findings support the concern that repeated sub-lethal exposure to biocides not only promotes adaptive resistance but also confers a decreased sensitivity to antibiotics.

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O 67 TETRACYCLINE RESISTANCE GENES IN SALMONELLA FROM GROWING PIGS AND THEIR RELATIONSHIP TO ANTIMICROBRIAL USE AND RESISTANCE TO OTHER ANTIMICROBIALS.

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Summary: The aim of this study was to describe the occurrence of three genes coding for tetracycline resistance in Salmonellae isolated from normal slaughter weight pigs, and to test for relationships between the occurrence of these genes, phenotypic resistance, and the use of antimicrobials in feed and water. Salmonella (1,431) were cultured at slaughter or just before slaughter among slaughter-age pigs, and were isolated using conventional methods. Three tetracycline resistance genes were