Portuguese piggeries as reservoir of antibiotic multi-resistant enterococci

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INTRODUCTION

• Dissemination of antibiotic resistance in community and hospitals has been facilitated by the extensive use of different antibiotics in clinical practice as well as in veterinary, husbandry and agriculture. Evidence of spread of antibiotic resistant bacteria to humans via food chain or by contact with animals led to the ban of antibiotics as growth promoters in the European in 1997. However, different European countries, as UK and Denmark, have reported an increase use of these molecules as therapeutic agents in order to reduce of animal diseases (Phillips et al., 2004).

• Beside food chain, piggeries have been reported to also contribute to environment contamination, such as soil, fresh vegetables and underground water, through land application of manure, sludge and wastewater and consequently by airborne dissemination of antibiotic resistant bacteria (Samaha et al., 2006).

• Antibiotic resistant strains may be co-selected by a diversity of biocides substances that include different antibiotics, antiseptics or metals which are given in food or be present in the animal production environment. This occurs because genes coding for resistance to different compounds are often located in the same genetic platform (Hassan et al., 2006).

• Recent Portuguese data revealed that, between 2004 and 2006, 632 tons of antibiotics were consumed by production animals being tetracyclines the most used (around 40 tons in 2006) (INFARMED, 2007).

• In order to evaluate the role of Portuguese piggeries as reservoir and contributor to environment dissemination of antibiotic multi-resistant enterococci, our goal included the detection of multi-resistant phenotypes, the characterization of antibiotic resistance genes and their ability to be involved in transfer events.

METHODS

• Bacterial strains and sample processing. Animal and environmental samples were collected in 5 intensive (A, B, C, E, F) and 1 extensive (D) production piggeries between April 2006 and December 2007 in the North, Centre and South of Portugal. Eighty six samples were classified in 5 groups concerning their nature: (i) pigs (n=21; faeces, nostril/surface swabs); (ii) food/meat (n=22: food, water, medicine, antiseptics) (iii) residues (n=17: waste lagoons, residual waters, manure, septic tanks); (iv) piggeries facilities (n=23: air, water/foodst, dust, soil); (v) river receiving piggeries effluents (n=1).

• Samples were enriched in peptone water (24h; 37ºC) and plated on Stainer-Stauffer with (16 μg/ml tetracycline, 1000μg/ml streptomycin, 125μg/ml gentamicin, 8μg/ml erythromycin, 16μg/ml ampicillin) and without antibiotics. One colony from each morphology and resistance phenotype was selected for further studies.

• Antibiotic susceptibility. Susceptibility to 12 antibiotics of different families was determined by the disk diffusion methods following CLSI guidelines (CLSI, 2007).

• PFGE. Fingerprinting of Enterococci strains was performed by PFGE for 26 representative (16 E. faecium and 12 E. faecalis) strains with different antibiotic resistance phenotype from 3 intensive production farms. Clonal relationships were established following the criteria previously described (Novais et al., 2005).

• Conjugation experiments were done in 67 tetracycline, 10 erythromycin and 6 gentamicin resistant isolates by filter mating methods at a 1:1 donor-recipient ratio, using the rifampicin and fusidic acid resistant E. faecium JH2-2, E. faecalis BM4105 or E. faecalis GE-1 as recipients. Transconjugants were recovered in selection plates with 60μg/ml of rifampicin plus 6μg/ml of tetracycline, 8μg/ml of erythromycin or 500 μg/ml of gentamicin after 48h of incubation (37ºC).

• Enterococcus faecalis clonal groups were confirmed by restriction to rifampicin, fusidic acid and tetracycline, erythromycin or gentamicin (120μg) by the disc diffusion method.

RESULTS

• For hundred and seventy-three enterococci were identified as E. faecium (n=171), E. faecalis (n=78), E. hirae (n=73), E. gallinarum (n=14); E. casseliflavus (n=5) and Enterococcus spp (n=132).

• Among all species, more than 50% of isolates were non-susceptible to tetracycline, minocycline, erythromycin or quinupristin-dalfopristin (Fig1). Antibiotic resistance to gentamicin, ampicillin, chloramphenicol was only detected in piglets with intensive production.

• IS1216 (63%), including 9 susceptible isolates), tetK (53%, including 4 susceptible isolates) and ermA (50%, including 4 susceptible isolates) were observed in a high number of strains (Fig2). Absence of tet or erm genes tested were observed in 13% and 19% of tetracycline or erythromycin nonsusceptible enterococci, respectively. (6p)-Ie-aph(3')-IIIa was detected in most of gentamicin nonsusceptible isolates tested and 79% of them presented simultaneously aph(3')-IIIa genes were observed in 5. E. faecium (5 piggery facility samples from 2 farms of intensive and 1 of extensive production) and 1. E. faecalis (1 faeces sample from 1 farm with intensive production).

• Forty-one resistance genes were observed and the ten most predominant were harbored by 76% of the isolates. They were scattered in different piggeries, species and sample types that included those such air, manure to be used in agriculture, animal food and water disinfected with UV and chlorine.

• Representative strains of most genotypes could transfer by conjugation tetracycline, macrolide and/or aminoglycoside resistance (Table 1).

• A polycyclic population was present (11 PFGE types for E. faecium and 9 for E. faecalis), although common clones were detected in different samples of the same farm (Manure and waste lagoons of B farm: 1. E. faecalis and 1 E. faecium clones; Piggeries, animal drinking water, dust of maternity of C farm: 1. E. faecalis clone; 2 different faeces samples of different animals from C farm: 1. E. faecalis clone).

Table 1, Percentage, species distribution, samples, piggeries and mating assays of predominate antibiotic resistance genotypes

<table>
<thead>
<tr>
<th>Enterococcus Group</th>
<th>Species</th>
<th>Sample</th>
<th>Piggeries</th>
<th>Mating Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecalis</td>
<td>E. faecalis</td>
<td>n=78</td>
<td>B,C,F</td>
<td>–</td>
</tr>
<tr>
<td>E. faecium</td>
<td>E. faecium</td>
<td>n=171</td>
<td>A,B,C,D,E</td>
<td>–</td>
</tr>
<tr>
<td>E. hirae</td>
<td>E. hirae</td>
<td>n=73</td>
<td>A,B,C,D,E,F</td>
<td>–</td>
</tr>
<tr>
<td>E. casseliflavus</td>
<td>E. casseliflavus</td>
<td>n=5</td>
<td>C</td>
<td>–</td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td>Enterococcus spp</td>
<td>n=132</td>
<td>A,B,C,D,E,F</td>
<td>–</td>
</tr>
</tbody>
</table>

• Positive mating assays. – Negative mating assays. ** Different isolates presented distinct results in mating assays. ND: Not determined.

CONCLUSION

• Antibiotic multi-resistant enterococci were commonly detected among Portuguese piggeries probably reflecting the antibiotic use in veterinary medicine. The presence of such strains in samples such manure used in agriculture and in air, is of concern since they can be spread outside piggery frontiers to environment and consequently to other animals and humans.

• The presence of a polycyclonal population associated to the ability of isolates to transfer different genes by conjugation supports that horizontal transfer events might have an important role in antibiotic resistant dissemination in Portuguese animal setting.

• The absence of tetracycline or erythromycin resistance genes in nonsusceptible phenotypes suggests that other resistant mechanism less common in enterococci might be present.

• Whether persistence of these antibiotic resistant isolates is only due to selection by antibiotics or also by other biocide agents used in pigs environment deserves further studies in order to better understand the epidemiology of antibiotic resistance in the animal setting of our country and to decrease the presence of such strains.

REFERENCES

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