Comparison of organic and conventional pig productions on prevalence, antibiotic resistance and genetic diversity of *Escherichia coli*

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Abstract

The objectives of this study were to assess the prevalence, the tetracycline resistance level and the genetic diversity of *Escherichia coli* isolated from organic pigs in comparison with conventional pigs. This study is integrated in a global European project, Safeorganic, funded trough CORE Organic II call. 25 organic and 25 conventional herds were considered in one slaughterhouse from April to October 2012. Colon content of 2 pigs per herds was sampled. For each pig, enumeration of *E. coli* and of tetracycline resistant *E. coli* (TET^+*E. coli*) was determined. Level of tetracycline resistance was then calculated. Isolates were typed by PFGE using *Xba*I enzyme. *E. coli* was detected for all the organic (n=50) and conventional pigs (n=50). TET^+*E. coli* was detected for 49 organic (98%) and 48 conventional pigs (96%). Number of *E. coli* per gram of colon content were significantly higher (p=0.0033) for conventional (6.81 $\log_{10}$UFC/g) than for organic pigs (6.19 $\log_{10}$UFC/g) as well as number of TET^+*E. coli* (p=0.00021) with 6.33 $\log_{10}$UFC/g for conventional pigs and 5.68 $\log_{10}$UFC/g for organic pigs. Finally, the level of tetracycline resistance was significantly higher (p=0.0033) for conventional (57.4%) than for organic pigs (37.9%). PFGE was done on 373 *E. coli;* they were distributed in 277 PFGE profiles. The genetic diversity was very high (*D value*=0.998). No pulsotype was common between organic and conventional pigs. Difference for the level of tetracycline resistance between organic and conventional pigs suggests that farm managements may have an impact on the amount of *E. coli* excreted and on their antibiotic resistance. However, it is difficult to estimate the impact on human health with 0.65 $\log_{10}$UFC/g difference between the two productions. Diversity of strains is so high that it is difficult to associate strains to a production.

Introduction

Spread of antibiotic resistance along the food-chain is a major food safety concern due to the risk of treatment failure of human foodborne infections. Reports suggest that organic animals carry lower levels of antibiotic resistant bacteria in faeces, including lower levels of multiresistant bacteria (Nulsen *et al*., 2008; Young *et al*., 2009). This is, however, scarcely documented, particularly for swine. Organic pig production differs in many aspects from the conventional pig farming. Several of these differences may influence the pattern of the microbiological flora including the pattern of antimicrobial resistance. For example, prophylactic use of antimicrobials and growth hormones is prohibited, although antimicrobials can be used to treat ill animals when all other options fail. There are also differences in feeding regimes, a lower animal density and access to outdoor areas (Bonde & Sørensen, 2004).

As the food animal production is a primary reservoir for foodborne pathogens and antibiotic resistance, there is a continuous need to regularly document the status in the primary production. Caecal/colon content, sampled at slaughter, can be used to characterize the herd status in relation to antibiotic resistance (Rosenquist *et al*., 2009). The bacterial population of these samples can reflect those originating from the farm and particularly *Escherichia coli* commonly used as indicator of faecal contamination and antimicrobial resistance. Thus, it may be used to compare antimicrobial resistance among populations. In the other hand, genetic profiles may be used to characterize also *E. coli* populations. Greater genetic diversity can be expected for organic animals due to the presumable lower antibiotic selection pressure and access to an open area.

The aims of this study were to assess the prevalence, the tetracycline resistance level and the genetic diversity of *E. coli* isolated at slaughter from colon of organic pigs in comparison with conventional pigs. Tetracycline was used as resistance indicator, as resistance to this antimicrobial is the more frequent for *E. coli* (EFSA 2012).

Material and Methods

Sampling

To evaluate the bacteriological status of the pigs at herd level, sampling of colon content from pigs was done at slaughter. Sampling was realized in one slaughterhouse from April to October 2012. In total, 25 organic herds and 25 conventional herds were considered; at each visit, and at each organic herd sampled, a conventional herd was also sampled. Colon content of 2 pigs per herd was collected at the evisceration step. Finally, 50 organic pigs and 50 conventional pigs were analyzed.

Enumeration of total E. coli and tetracycline resistant E. coli

Ten to 25 g of each colon content were diluted in 90 ml of tryptone salt (AES Chemunex, Bruz, France) and a 1:10 serial dilution was realized until the dilution -6. One ml of each dilution was dropped on a 3M™ Petrifilm™ Select *E. coli* Count Plate (SEC plate) (3M, Cergy-Pontoise, France). Characteristic colonies were counted in order to determine the quantity of *E. coli* per gram of colon content.
In parallel, and as described by Wu *et al*. (2008), 1 ml of each dilution was supplemented with oxytetracycline for a final concentration of 64mg/L and dropped on a 3M™ Petrifilm™ Select *E. coli* Count Plate. Characteristic colonies were counted in order to determine the quantity of tetracycline resistant *E. coli* (TET+*E. coli*) per gram of colon content.
Level of tetracycline resistance for each sample was then determined by the percentage of TET$^+$*E. coli* from the total number of *E. coli*.
A multiplex-PCR was performed (Perrin-Guyomard *et al*., 2008) to confirm isolates as *E. coli*.

Statistical Analysis

Statistical analyses were performed using SAS software. The variables to be explained were enumeration (log_{10} UFC *E. coli* total/g, (log_{10}T) and log_{10} UFC *E. coli* resistant/g (log_{10}R)) and the resistance level (%_UFCR). Test used was Anova when possible or Wilconxon otherwise. To determine if differences observed between results were significant, a variance analysis was performed.

PFGE typing and analyzes of the genetic profiles

When possible, two isolates per positive 'SEC plate' and positive 'SEC plate+TET' (4 per sample) were characterized by RFLP-PFGE using *Xba*I enzyme (Ribot *et al.,* 2006). *Salmonella enterica* serotype Braenderup H9812 was used as molecular size marker (Hunter *et al*., 2005).
*Xba*I profiles were analyzed on BioNumerics® software (V 6.5, Applied Maths, Kortrijk, Belgium). The similarities between profiles, based on the position of the restricted fragments, were calculated using the coefficient of Dice with a maximum tolerance of 1% (Struelens, 1996). The Simpson's index (Hunter, 1990) was calculated to estimate the diversity of the sample.

Results

From colon content, on the 100 sampled pigs, *E. coli* was detected for all the organic pigs (n=50) and conventional (n=50). TET$^+$*E. coli* was detected for 49 organic pigs (98%) and 48 conventional pigs (96%). The number of *E. coli* per gram of colon content were significantly higher (6.81 log_{10}UFC/g) for conventional pig (Table 1) than for organic pigs (6.19 log_{10}UFC/g) (p=0.0033). A significant difference for the number of TET$^+$*E. coli* per gram of colon content was also observed between organic (5.68 log_{10}UFC/g) and conventional pigs (6.33 log_{10}UFC/g) (p=0.00021). The level of tetracycline resistance is higher for conventional pigs (57.4%) than for organic pigs (37.9%); this is also significant (p=0.0033).

Table 1: Enumeration of total E. coli and TET$^+$E. coli from colon content of organic and conventional pigs and level of tetracycline resistance.

Type of production	Total *E. coli*		TET^{+}*E. coli*		Level of tetracycline resistance
	N° of positive samples (%)	log_{10}UFC/g	N° of positive samples (%)	log_{10}UFC/g	
Organic	50 (100%)	6.19	48 (98%)	5.68	37.9%
Conventional	50 (100%)	6.81	49 (99%)	6.33	54.4%

A total of 373 *E. coli* and TET+*E.coli* isolates were collected from colon content, 195 from organic pigs and 178 form conventional pigs (Table 2). Isolates were distributed in 277 PFGE profiles after *Xba*I restriction: 142 for isolates from organic pigs and 135 from conventional pigs (Table 2). For individual sample, the diversity ranged from 0.25 (one subtype found among 4 isolates) to 1.00 (4 subtypes found among 4 isolates).
No PFGE profile was common to the two type of production, and, no specific genetic cluster was observed within the dendrogram, even using a cut-off value of 80% genetic similarity.
Thus, the diversity index was very high whatever the type of production (ID≥0.99). Most of the time, isolates harboring a same PFGE profile where from a same sample or a same herd. Only 1 PFGE profile was common to isolates from 2 different organic herds sampled at different days.

Table 2: PFGE diversity after XbaI restriction

Type of production	Number of isolates	Number of PFGE profiles	Index of diversity [95% confidence intervals]
Organic	195	142	0.99 [0.99-1.00]
Conventional	178	135	0.99 [0.99-1.00]
Total	373	277	0.99 [0.99-1.00]

Discussion

E. coli was detected for all the pigs; these commensal bacteria colonize intestinal tract of almost warm-blood animals and are present throughout the environment. So, the access to an outdoor area of organic pigs may contribute to maintain a high level of contamination.
Difference for the level of tetracycline resistance between organic and conventional pigs suggests that farm managements may have an impact on the amount of *E. coli* excreted and on their antibiotic resistance. The lower level of tetracycline resistance for organic pigs could be explained by contamination in the outdoor area with wild strains supposed to be more sensitive to antibiotic and overall by the restricted antimicrobial practices in organic farm.
Difference in antibiotic resistance was previously observed by Tadesse *et al.,* (2011) between farms with or without antibiotic treatments. They observed a higher erythromycine resistance for *Campylobacter* in farm with antibiotic treatment. For our study, after contact with farmers, only one of the herds sampled in our study has been treated with antimicrobials which could explain our results. However, some studies in North America showed that antimicrobial resistance can remain in faecal *E. coli* population in pig decades after the drug was banned from food-animal production (Maynard *et al*., 2003).
The genetic diversity of *E.coli* was very high for each production and it was difficult to associate strains or genetic clusters to a production. PFGE demonstrated to be an interesting epidemiological tool to differentiate *E. coli* within a specific serotype, as O157:H7 (Watabe *et al*., 2008) or to differentiate *E. coli* isolates from piglets with diarrhea (Vu-Khac, *et al*., 2007). But finally, for the commensal *E. coli* population, this method cannot be used as origin population marker.

Conclusion

Difference between organic and conventional pigs suggests that farm managements may have an impact on commensal bacteria. Nevertheless, while difference in the level of tetracycline resistance between organic and conventional pigs was statistically significant, it is difficult however to estimate the impact on human health with a difference of 0.65 log_{10}UFC/g of *E. coli* tetracycline resistant between the two productions. Strains are being tested with a panel of antibiotics; the results will clarify those previously obtained.
PFGE is not a good tool to associate genetic profiles to production. Other typing methods should be considered.

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