Antimicrobial resistance in *Campylobacter* from pigs in French slaughterhouses

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SUMMARY

The prevalence of *Campylobacter* in pigs arriving in French slaughterhouses was studied. After direct isolation, 323/600 (53.8 %) faecal samples were positive for *Campylobacter*. 98.1 % isolates were identified as *Campylobacter coli*, 0.9 % as *C. jejuni*, and species could not be determined for three *Campylobacter* strains. The minimal inhibitory concentrations (MICs) of six antimicrobial agents were determined. 12, 20, 12, 83, 65 and 0 % of the *C. coli* strains were resistant respectively to ampicillin, nalidixic acid, ciprofloxacin, tetracycline, erythromycin and gentamicin. One of the *C. jejuni* strains was sensitive to the six molecules and the two other strains were categorised as intermediate for ampicillin, and resistant for tetracycline and erythromycin. No significant difference (p > 0.05) of resistant strains distributions according to antimicrobial agents administrated to pigs could be detected.

KEY-WORDS : antimicrobial - resistance - *Campylobacter* - pigs - slaughterhouses.

Introduction

*Campylobacter* are commonly incriminated in food-borne infections. The main source of human contamination seems to be chicken with *C. jejuni* as main isolated species. *C. coli* appears as the second species implicated in human campylobacteriosis [7]. Only few studies have reported data concerning the implication of swine production in campylobacteriosis. However, swine production seems to be a wide reservoir of *Campylobacter* with contamination level at slaughter age comprised between 47 and 95 % [8]. A recent study reported that the consumption of pork products may be at risk for *Campylobacter* infection [7].

A French monitoring study was organised in 1999 to evaluate the prevalence and the antimicrobial resistance of indicator (*Enterococcus, Escherichia coli*) and zoonotic (*Salmonella, Campylobacter*) bacteria isolated from broilers. In 2000, the surveillance programme was extended to the swine production.

Materials and methods

A) BACTERIAL SAMPLING

600 pig faecal samples were collected in ten French slaughterhouses by meat inspection staff. Data concerning antimicrobial consumption (coccidiostats, growth promoters or antimicrobial treatments) of the sampled herds were registered on the breeding document accompanying pigs to slaughterhouse. Only one pig per herd was included.
B) ISOLATION AND IDENTIFICATION

Faecal samples from pigs were weighted and diluted 1/10 in 25 % glycerol peptone broth (GPB) (AES). A 10 µl of this suspension was then streaked on Karmali (AES) and Butzler no.2 (Merck, Fontenay-sous-Bois, France) selective media. After 48 to 72 hours of incubation at 42°C under microaerobic atmosphere (Campypak H2CO2, AES, Combourg, France), one characteristic colony was selected and streaked on each medium and incubated for 24 to 48 hours before storing in GPB at -70°C. Three hundred and twenty-three randomly selected strains were thawed, streaked on Mueller-Hinton agar plate (AES) supplemented with 5 % sheep blood (MHSB), and incubated for 48 to 72 hours. For each MHSB plate, one characteristic colony was isolated on MHSB plate for purification, incubated for 24 to 48 hours and this culture was used for identification and antimicrobial susceptibility testing.

Identification of C. coli and C. jejuni species was realised by m-PCR [5].

C) ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was done according to the NCCLS document M7-A4. Six antimicrobials were tested: ampicillin (0.25 to 64 µg/ml) (Sigma, Saint-Quentin-Fallavier, France), nalidixic acid (1 to 256 µg/ml) (Sigma), ciprofloxacin (0.03 to 32 µg/ml) (Bayer, Puteaux, France), tetracycline (0.125 to 128 µg/ml) (Sigma), erythromycin (0.25 to 64 µg/ml) (Sigma) and gentamicin (0.03 to 16 µg/ml) (Sigma). Campylobacter strains were suspended in 4 ml Brucella broth, incubated at 37°C for 16-20 hours under microaerobic atmosphere, then adjusted to 0.5 McFarland and one µl of each suspension containing about 10^4 Colony Formit Units / spot was inoculated on each Mueller-Hinton plate with 5 % sheep blood plate supplemented with antimicrobial agent. Plates were incubated 48 h at 37°C under microaerobic atmosphere. Susceptibility categorisation for Campylobacter was carried out according to the statement 2000 of the Antiibiogram Committee of the French Society for Microbiology.

D) STATISTICAL ANALYSIS

Data concerning coccidiostats and antibiotic agents administered to animals were recorded in an Open Access 1997 Database as results of Campylobacter antimicrobial phenotypes. Pearson chi-square and Fisher’s exact tests were used for statistical analysis with a significant level of 5 %.

Results

A) PREVALENCE AND IDENTIFICATION OF CAMPYLOBACTER SPECIES

Among the 600 faecal samples, 323 were contaminated with thermod tolerant Campylobacter. According to the m-PCR method, 317 strains were identified as C. coli (98.1 %), 3 as C. jejuni (0.9 %) and 3 strains (0.9 %) could not be identified to the species level.

B) ANTIMICROBIAL SUSCEPTIBILITY

The distributions of MIC of C. coli strains are showed in figure 1. All strains were sensitive to gentamicin. C. coli strains were usually sensitive to ampicillin and, ciprofloxacin and nalidixic acid with only respectively 12, 12 and 20 % of resistant strains whereas 65 % and 83 % of the C. coli strains were respectively resistant to erythromycin and tetracycline. Tetracycline and erythromycin resistance phenotype was the main antimicrobial resistance phenotype encountered (38 % of C. coli strains). One of the three C. jejuni strains was sensitive to all antimicrobial agents tested and the other ones were resistant to erythromycin and tetracycline and intermediate to ampicillin.

C) DISTRIBUTION OF RESISTANT STRAINS ACCORDING TO ANTIMICROBIAL AGENTS ADMINISTERED TO FLOCKS

Percentages of antimicrobial resistant strains were analysed according to coccidiostats, antimicrobial treatments and additives administrated to animals. No significant difference (p > 0.05) was observed.

Discussion

Our results were based on direct streaking of a single faecal sample from pig herds arriving in slaughterhouses. A majority of Campylobacter coli strains were isolated from pigs faecal samples as previously reported in different countries [1, 6]. STUDAHL and ANDERSON (2000) showed that C. coli was isolated from pork products like chops and loin and consequently represented an infection risk for man.

In human Campylobacter infection, when treatment is needed, macrolides and fluoroquinolones are commonly used [3]. The occurrence of resistance is generally higher among C. coli compared to C. jejuni [2]. The percentage of erythromycin resistant C. coli strains was notably high in French pigs compared to C. coli strains isolated in 1999 in broiler productions. A similar observation was previously reported in several studies [2]. The reason of the high percentage of macrolide resistant C. coli from pigs was not determined. It could be related to a higher mutation rate of C. coli strains or to the selective pressure induced by the use of antimicrobial agents in farms [2]. Data collected by meat inspection staff concerning antimicrobial consumption of sampled pigs did not seem comprehensive and may have hidden eventual effect on the selection of resistant strains. It is important to remember that, in 1999, just before our sampling campaign, the European Union banned the use of tylosin as growth promoter, which had been largely used for years in animal feeds. Moreover, a recent epidemiological study about antibiotic prescription in pig therapy in France reported that tylosin was used in 24 % of prescriptions for digestive diseases behind colistin (35 %) and in 8 % of prescriptions for respiratory diseases behind tetracyclines (30 %), a feature that could explain the high percentages of tetracycline and/or erythromycin Campylobacter resistant strains [4]. The next pig sampling campaigns should reveal whether the ban of tylosin as growth promoter will affect the percentage of macrolide resistant bacteria.
Most \textit{C. coli} strains from pigs were sensitive to ciprofloxacin and fluoroquinolones should be effective to eradicate human infections as far as the strain origin is pig. Gentamicin was active for all tested strains and might be used in case of serious \textit{Campylobacter} infection. However, emergence of gentamicin resistant \textit{C. jejuni} and \textit{C. coli} in broilers and humans in Spain has been observed and should draw our attention [6].

These results are to our knowledge the first data concerning prevalence and antimicrobial susceptibility of \textit{Campylobacter} in French pig production. For \textit{Campylobacter} isolated from pigs, erythromycin is ineffective contrary to \textit{Campylobacter} isolated from broilers. Because pig is a potential source of human \textit{Campylobacter} contamination, it will be particularly interesting to monitor antimicrobial resistance of pig \textit{Campylobacter} as well as other zoonotic and sentinel bacteria in the next few years to evaluate long-term effect of the ban of antibiotics as growth promoters in swine production.

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\section*{References}


