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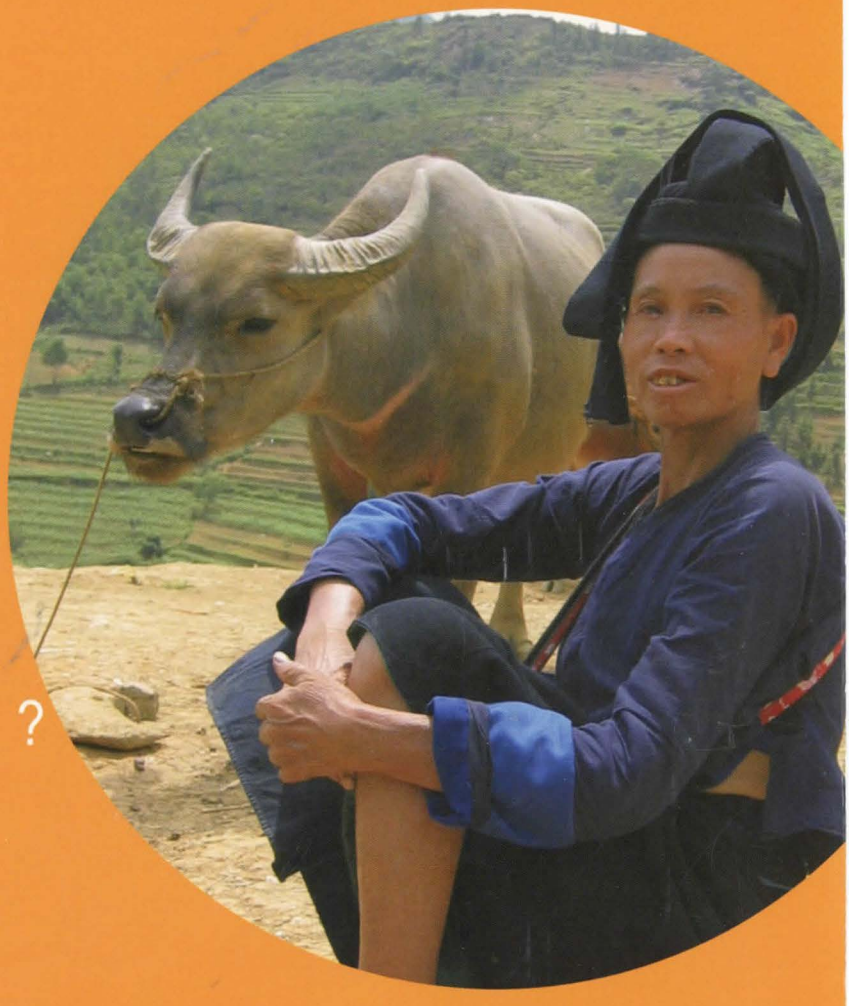

Does control of animal infectious risks offer a new international perspective ?

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GENETIC CHARACTERIZATION AND ANTIBIOTIC RESISTANCE OF *CAMPYLOBACTER* SPP. ISOLATED FROM POULTRY AND HUMANS IN SENEGAL

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ABSTRACT

The main objectives of this study were to investigate the diversity of *Campylobacter* genotypes circulating in Senegal and to determine the frequency of antibiotic resistance. Strains of *C. jejuni* isolated from poultry (n=99) and from patients (n=10) and *C. coli* isolated from poultry (n=72) were subtyped by pulsed-field gel electrophoresis (PFGE). The pulsotypes obtained revealed a significant genetic diversity in both species, but without any predominant pulsotypes. However, farm-specific clones were identified in the majority of poultry houses (76.5 %). Human and poultry isolates of *C. jejuni* had common PFGE patterns. High quinolone-resistance rates were observed for *C. jejuni* (43.4 %) and *C. coli* (48.6 %) isolates obtained from poultry.

INTRODUCTION

Thermophilic *Campylobacter* spp. are among the most important agents of human gastrointestinal infections in the developed and the developing world (Prasad *et al,.* 2001). Contaminated food is a common source of human illness and consumption or handling of poultry is considered to be a major route of infection. In Senegal, *C. jejuni* and *C. coli* have been recovered from commercial poultry (Cardinale *et al.,* 2004). However, their involvement in human infections remains unknown, as does the significance of chicken meat as a vehicle of infection, since no

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epidemiological analysis has been made previously in that country. The objectives of the present study were to (i) investigate the diversity of genotypes occurring on poultry farms and in broiler chicken meat in Dakar, and to compare these to clinical isolates collected from the same area; (ii) evaluate the frequency of antibiotic resistance in poultry isolates and determine whether resistance is associated with specific genotypes.

MATERIALS AND METHODS

Bacterial isolates

The poultry isolates of *C. jejuni* and *C. coli* originated from farms and retail shops and were collected between January 2000 and December 2003 in the Dakar (capital city) region. Forty broiler farms were chosen at random. These farms belonged to the modern poultry sector and all showed similar characteristics. At each farm, five broilers were slaughtered and breast-skin samples (each weighing *ca* 25 g) were taken. Farm managers were asked to complete a questionnaire about any drugs used during the life-span of the flock. In addition, 10 randomly selected retail shops were investigated and skin samples taken from five chicken carcasses in each case. The samples were analysed as described by Cardinale *et al.,* (2004). Ten human isolates, obtained from epidemiologically-unrelated domestic patients during 2001 and 2002, were provided by the Pasteur Institute of Dakar. These originated from faecal samples, cultured on plates of *Campylobacter* blood-free selective medium and identified as described previously.

PFGE analysis

It was done as described by Liebana *et al.* (2001) with two macrorestriction enzymes.

Antibiotic Resistance

The breakpoints were those recognised by the Antimicrobial Committee of the French Society for Microbiology. Breakpoints for resistance susceptibility were respectively $>16\ mg.l^{-1}$ for amoxicillin (Am), $>16/2\ mg.l^{-1}$ for amoxicillin-clavulanic acid (Ac), $>4\ mg.l^{-1}$ for erythromycin (E), $>16\ mg.l^{-1}$ for nalidixic acid (Na) and $>2\ mg.l^{-1}$ for ciprofloxacin (Ci).

Determination of mutations in the quinolone resistance determining region (QRDR) of the gyrA gene

26 nalidixic acid- and ciprofloxacin-resistant, and two nalidixic acid-resistant and ciprofloxacin-susceptible strains of *C. jejuni* from poultry were analysed. Chromosomal DNA was extracted from each strain by boiling, as described previously (Bachoual *et al.* 2001).

RESULTS

Campylobacter spp. were isolated from 68.4 % of the poultry samples. The contamination rate was similar at both types of test location (69 % at the farms *vs.* 66 % at the retail shops). Four farms and four retail shops harboured both *C. jejuni* and *C. coli*. The species distribution was *C. jejuni* 57.9 % and *C. coli* 42.1 %. Only ten human isolates were examined; all belonged to *C. jejuni*.

PFGE Analyses

For *Campylobacter jejuni, t*wenty-five pulsed-field profiles were obtained with both enzymes. None of these patterns indicated a predominant genotype. Most of the human isolates (40 %) were grouped in pattern SJ6KJ6. The dendrogram showed several clusters but only one was significant (18 isolates). For *Campylobacter coli, n*ineteen pulsed-field profiles were obtained with both enzymes. There was no predominant genotype. The dendrogram showed several clusters that gathered together only a few isolates. Most of the farms (76.5 %) harboured a unique genotype.

Antibiotic resistance

For *Campylobacter jejuni,* 38.4 % were susceptible to all the antibiotics tested. 43.4 % were resistant to the quinolones. The most commonly observed resistance pattern was Am Na Ci, accounting for 22.2 % of the isolates. This antimicrobial resistance profile was also seen in two of the human isolates. For *Campylobacter coli, o*nly 16 isolates (22.2 %) were fully susceptible to the antibiotics tested and 35 (48.6 %) were resistant to the quinolones. Am Na Ci was the most common pattern, accounting for 36.1 % of the isolates. Eight isolates (11.1 %) were each resistant to four of the drugs.

The antimicrobial resistance patterns were not related specifically to the PFGE patterns.

Resistance to quinolones was found in isolates from most of the farms (85 %) that used quinolones to treat poultry during the rearing period.

Sequence analysis of the QRDR of the gyrA gene

Among the highly quinolone-resistant strains of *C jejuni* (ciprofloxacin MIC 8- >32 $mg.l^{-1}$, nalidixic acid MIC 32- >256 $mg.l^{-1}$), 18 showed the Threonine86-Isoleucine substitution, four the Threonine86-Alanine substitution and four showed no mutation in the QRDR. Two strains with low-level resistance to nalidixic acid (MIC = 16 $mg.l^{-1}$), but susceptible to ciprofloxacin, had the Threonine86-Alanine substitution.

DISCUSSION

In this study, we identified a significant diversity of PFGE patterns among strains from different farms, but with specific clones occurring in most of the farms. The presence of one predominant strain at a particular farm has already been demonstrated in several studies (Jacobs Reitsma 1997). In fact, studies on the dynamics of infection in experimentally-infected chicken flocks have concluded that some strains of *Campylobacter* are able to become dominant, while preventing colonisation by other strains. In Senegalese poultry production, numerous sources of infection exist and a previous study has highlighted several possible risk factors (Cardinale *et al,.* 2004). Poultry flocks appear to become infected mainly by horizontal pathways via the farm environment. This is consistent with earlier reports showing that the main source of infection is likely to be the immediate surroundings of the house, in spite of the use of hygiene barriers (Van de Giessen *et al.,* 1998). By contrast, the samples from retails shops harboured a variety of strains. In Senegal, retail outlets are small shops that sell only a relatively low number of chickens and are supplied by numerous farms. Thus, each shop is a site of possible cross-contamination from chickens of multiple origins. In this study, the human and poultry isolates of *C. jejuni* shared common PFGE patterns but because of the low number of human isolates, it is difficult to draw firm conclusions about the potential for transmission from broiler chickens to humans, as is the case in other countries (Petersen *et al.,* 2001). In Senegal, many enteritis sufferers do not seek medical attention and, even among those that do, only some will

have a stool specimen cultured for enteric pathogens. This is especially true for *Campylobacter*, because there is no systematic investigation of *Campylobacter* cases in medical laboratories. Further investigation of human isolates is badly needed. In our survey, high rates of resistance to quinolones were observed for both species of *Campylobacter* isolated from chicken samples. This was similar to the results obtained in several European countries and in Japan (Chuma *et al.,* 2001). The observed resistance was probably generated *de novo* by genetic modification from the selective pressure of antibiotic usage. Most of our highly-quinolone-resistant strains exhibited the Threonine86-Isoleucine substitution, but we also found a Threonine86-Alanine substitution in four isolates and no mutation in four others. The Thr-86-Ala change was described previously by Bachoual *et al.* (2001) for one clinical strain of *C. jejuni* with high-level resistance to nalidixic acid (MIC 64 $\mu g.ml^{-1}$), but low-level resistance to ciprofloxacin (MIC 2 $\mu g.ml^{-1}$). However, we believe that other mutational changes might exist in *gyrA* outside the critical region. It has been shown that factors other than *gyrA* QRDR mutations, such as efflux pumps and *parC*, may contribute to the resistance phenotype (Lin *et al.,* 2002).

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