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Campylobacter spp. – Prevalence on pig livers and antimicrobial susceptibility

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ABSTRACT

The objective of the study was to determine the prevalence of *Campylobacter* spp. on surfaces of slaughtered pig livers. Multilocus sequence typing (MLST) was performed to determine the sequence types (STs) of selected *Campylobacter coli* isolates. Additionally, *C. coli* and *Campylobacter jejuni* isolates were tested for antimicrobial susceptibility by the broth dilution method. The minimal inhibitory concentrations were determined for erythromycin, gentamicin, ampicillin, ampicillin/sulbactam, nalidixic acid, ciprofloxacin, tetracycline and trimethoprim/sulphamethoxazole.

Samples were taken during the slaughtering process in a slaughterhouse in Lower Saxony, Germany. Altogether, 10% of 1500 surfaces of pig livers from 50 fattening herds was found to be *Campylobacter* positive, with *C. coli* as the predominant species (76%) followed by *C. jejuni* (21%). Resistance to erythromycin and tetracycline was higher in *C. jejuni* compared to *C. coli*, whereas *C. coli* were more resistant to quinolone compared to *C. jejuni*. Fluoroquinolone resistance is usually associated with cross-resistance to quinolone, but in the presented investigation *C. coli* as well as *C. jejuni* showed a higher resistance to ciprofloxacin (28.6% and 20.0%, respectively) than to nalidixic acid (9.5% and 0%, respectively).

A high genetic diversity of the *C. coli* isolates was demonstrated by MLST. Differences in STs and antimicrobial resistance pattern indicate that the *Campylobacter* strains originated from the pig itself and not from the slaughterhouse. A comparison of the STs with those reported in the *C. jejuni/coli* PubMLST database showed an overlap of porcine and human isolates, indicating that *C. coli* isolates from pigs should be considered as potential sources of human infection.

1. Introduction

Campylobacteriosis is recognized as the leading cause of human bacterial infectious intestinal disease in the EU (EFSA, 2011). Consumption of food of animal origin is considered a major source of infection. In Germany, 71,307 human cases were reported in 2011 (Robert-Koch-Institut, 2012). The annual incidence of *Campylobacter coli* infection in humans was found to be 18.6% (Gurtler et al., 2005). Animals such as pigs, cattle, and poultry are potential reservoirs for the bacteria. Although *Campylobacter jejuni* is predominant in poultry and cattle, it is infrequent in pigs, in which *C. coli* predominate (Nielsen et al., 1997).

The bacterium is highly prevalent in the intestinal tract of pigs arriving at the slaughter facility, but it is seldom

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detected on carcasses after overnight chilling, due to the sensitivity of the bacterium to freezing and drying (Pearce et al., 2003). Thus pork is not considered to be a significant source of human campylobacteriosis in industrialized countries (De Jong et al., 2009). Nonetheless, a Swedish case-control study demonstrated that consumption of pork meat with bones, like pork loin, was a risk factor for *Campylobacter* infection (Studahl and Andersson, 2000) and in the UK, *Campylobacter* were isolated in 18.3% of edible offal samples (liver, heart, kidney, and tripe) of porcine origin collected at the point of sale (Little et al., 2008).

Discrepancies in the antimicrobial resistance quotas, especially of erythromycin, among *C. coli* isolates originating from poultry and humans support the hypothesis that at least some of the resistant *Campylobacter* strains causing infection in humans can be related to sources other than poultry products (Luber et al., 2003). *C. coli* isolates from pigs are frequently resistant to erythromycin and tetracycline (Thakur and Gebreyes, 2005). The ability of *C. coli* to exhibit resistance to multiple antimicrobials including fluoroquinolones and macrolides more frequently than *C. jejuni* has raised further interest from a public health perspective (Thakur and Gebreyes, 2005; Little et al., 2008), because these are the antimicrobials which are recommended for systemic *Campylobacter* infections especially in immunosuppressed patients and infections which are severe and longlasting.

Microbiological typing techniques can allow isolates to be grouped on the basis of genotype, potentially enabling the identification of host-associated lineages from possible food chain sources (Sheppard et al., 2009). Multilocus sequence typing (MLST) is the method of choice to study molecular epidemiology of *Campylobacter* (Dingle et al., 2001; Miller et al., 2005).

In the presented study, surfaces of livers from slaughtered pigs were chosen as a means of estimating the risk of consumers being infected with pathogenic *Campylobacter* spp. strains from offal of porcine origin. Selected isolates were used to determine the minimal inhibitory concentrations (MICs) of eight antimicrobial agents. Furthermore, MLST was performed for comparing sequence types (STs) with those reported in the *Campylobacter* database (http://pubmlst.org/campylobacter).

2. Materials and methods

2.1. Sample collection

Between July 2007 and June 2008, 1500 surfaces of slaughtered pig livers from 50 conventionally housed fattening herds were swabbed while hanging on a hook during the slaughtering process. The slaughterhouse, which processes about 450 pigs/h, was located in Lower Saxony, Germany. Pigs were slaughtered with about 110 kg slaughter weight. The herds belonged to an organization of pig producers. Herd size varied between 185 and 3,100 pigs. Criteria for selecting the herds was a mean batch size of 50 pigs per delivery to the slaughterhouse at minimum, to be able to take all liver samples from a herd on one day. Livers were swabbed immediately after evisceration and before veterinary inspection. The samples, 30 swabs from each herd, were taken from an area of about 10 cm × 10 cm and stored in Cary-Blair medium (BBL Cultureswab Cary Blair-Medium, Firma Becton und Dickinson, Heidelberg, Germany). Swabs were transported at 4 °C to the laboratory for analysis within 24 h.

2.2. Isolation

Swabs were transferred into tubes with 2 ml Bolton broth (Oxoid, Wesel, Germany) for selective enrichment. After incubation for 24 h at 37 °C, a loopful of the broth was streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid, Wesel, Germany) and incubated for 48 h at 37 °C, microaerobically using Anaerocult C (VWR, Darmstadt, Germany) to generate an O_2-deficient, CO_2-enriched atmosphere in anaerobic jars (VWR). Presumptive *Campylobacter* colonies were subcultivated microaerobically on mCCDA again for 24 h at 37 °C.

2.3. Identification

DNA was extracted from one selected *Campylobacter* colony per sample (DNA Genomic Purification Kit, Amersham Bioscience, Freiburg, Germany). The identity was verified by polymerase chain reaction (PCR). Specific primers were used for the genus *Campylobacter* (Van Doorn et al., 1998), and the species *C. coli* (Gonzalez et al., 1997) and *C. jejuni* (Marshall et al., 1999).

2.4. Determination of the MIC

Thirty *Campylobacter* isolates were randomly selected and MICs were determined by the broth dilution method according to CLSI Standards (NCLLS, 2002). The colonies (*C. coli* ($n = 20$) and *C. jejuni* ($n = 10$)) were removed from the freezer, inoculated into 5 ml of Mueller–Hinton broth (CM 405; Oxoid) and incubated for 24 h at 37 °C in a microaerophilic atmosphere. Susceptibility testing inoculums (5–7 log CFU/ml) were prepared by transferring 0.15 ml of each culture into 10 ml of fresh Mueller–Hinton broth. Each well of the Sensititre®plate containing an antibiotic gradient of eight different antibiotics was filled with 100 μl inoculum, and the plates were sealed with film and incubated at 37 °C under microaerophilic conditions. The panels were inspected 24 h later for visible growth. The MIC was defined as the lowest concentration to prevent visible growth.

The following is a list of antimicrobial agents or antimicrobial combinations and ranges of concentrations used: erythromycin (0.0078–16 mg/L), gentamicin (0.0078–32 mg/L), ampicillin (0.0156–32 mg/L), ampicillin/sulbactam (0.0156/0.0078–32/16 mg/L), nalidixic acid (0.0156–32 mg/L), ciprofloxacin (0.0078–16 mg/L), tetracycline (0.0078–16 mg/L), and trimethoprim/sulphamethoxazole (0.0078/0.1484–16/304 mg/L). As the CLSI breakpoint interpretative criteria for *C. jejuni/coli* were adapted to those for *Enterobacteriacea* and were only given for erythromycin, tetracycline, doxycycline, and ciprofloxacin, breakpoints for *Enterobacteriaceae* were used for all the antimicrobials except as recommended by CLSI. According to the CLSI interpretative