Food Microbiology 34 (2013) 130–136

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Molecular characterization and antibiotic resistance profiling of *Campylobacter* isolated from cattle in Polish slaughterhouses

Kinga Wieczorek [a,*], Edyta Denis [a], Orla Lynch [b], Jacek Osek [a]

[a] *Department of Hygiene of Food of Animal Origin, National Veterinary Research Institute, Partyzantow 57, 24-100 Pulawy, Poland*
[b] *Teagasc Food Research Centre-Ashtown, Ashtown, Dublin 15, Ireland*

ARTICLE INFO

Article history:
Received 24 March 2012
Received in revised form
31 July 2012
Accepted 7 December 2012
Available online 14 December 2012

Keywords:
Campylobacter
Cattle
Antimicrobial resistance
Genetic characterization

ABSTRACT

A total of 812 samples from bovine hides and the corresponding carcasses collected at the slaughterhouse level in the eastern part of Poland were examined for the presence of *Campylobacter jejuni* and *Campylobacter coli*. Recovered isolates were confirmed using species-specific PCR, characterized by the presence of 11 putative virulence genes and antimicrobial susceptibility was determined using a microbroth dilution method. Furthermore, the genotypic relatedness of the isolates was determined by PFGE profiling and virulence pattern cluster analysis. The prevalence of *Campylobacter* was 25.6% and 2.7% in bovine hide and carcass samples, respectively. The presence of virulence markers varied between *C. jejuni* and *C. coli* species however, the majority of strains possessed the *cadF*, *flhA*, *flaA* genes, irrespective of the bacterial species and origin. The lower number of the strains was positive for the invasive associated markers – *virB11* and *wlaN*. Antibiotic profiling showed that campylobacters were most frequently resistant to quinolones and fluoroquinolones (nalidixic acid and ciprofloxacin, 38.3% of each, respectively) followed by streptomycin (24.3%) and tetracycline (20.9%). Resistance to erythromycin and gentamicin was demonstrated in 4.3% and 2.6% of strains, respectively. Comparisons of the PFGE and virulence marker profiles of the isolates reflected the high genetic diversity of *Campylobacter* tested. Moreover, a poor correlation between the PFGE type, pathogenic gene marker and antimicrobial resistance patterns was observed.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Thermophilic *Campylobacter* are recognized as one of the most common causes of food-related gastroenteritis in humans (Anon, 2011a). The pathogen acts as a commensal in many food-producing animals such as cattle and poultry, constituting an important food-borne source of human infections. There are few information concerning the contamination of cattle carcasses at the slaughterhouse level (Bae et al., 2007; Englen et al., 2007) and such study has never been performed in Poland. According to the European Food Safety Authority (EFSA) report, campylobacteriosis in the European Union is still the most common foodborne bacterial disease with 198,252 cases and notification rate of 45.57 per 100,000 population in 2009 (Anon, 2011a). In Poland at the same time only 357 confirmed cases were notified with the incidence rate of 0.94 (Anon, 2011a). There is no information how many of these infections were attributed to *Campylobacter* of cattle origin.

Although several putative pathogenic markers have been described in *Campylobacter* isolates, the pathogenesis of infection is still not yet well defined (Müller et al., 2006; Fernandes et al., 2010). However, flagella-mediated motility (determined by the *flaA* and *flhA* genes), adherence to intestinal epithelial cells (*cadF* and *docA* gene products), invasion and survival in the host cells (*ciaB*, *iam*, *wlaN*, and *virB11* markers) as well as the ability to produce toxins (*cdt* genes) are important virulence factors involved in campylobacteriosis (Bang et al., 2003; Datta et al., 2003; Fernandes et al., 2010).

It has been observed an increasing trend of antibiotic resistance in *Campylobacter* isolates, especially multi-drug resistance among these microorganisms within the food chain. Some antimicrobials, such as quinolones and macrolides are considered of the highest concern because of their significance in human medicine (Smole Možina et al., 2011). Although the majority of human *Campylobacter* infections are sporadic and cause by non-related strains (Anon, 2011a), several molecular typing systems allow to identify and differentiate isolates from different sources. Moreover, identification of these isolates provides the ability to study the pathogenesis of infections, investigate way of transmission, and assist

* Corresponding author. Tel.: +48 81 8893185; fax: +48 81 8862595.
E-mail address: kinga.wieczorek@piwet.pulawy.pl (K. Wieczorek).

0740-0020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2012.12.003

with surveillance and prevention of campylobacteriosis in humans (Eberle and Kiess, 2012). Tracing the sources and understanding the epidemiology of *Campylobacter* infections is increasingly relying on molecular typing. Pulsed-field gel electrophoresis (PFGE) is a highly discriminatory technique which has been extensively used for the molecular typing of thermophilic *Campylobacter* (Ribot et al., 2001; Praakle-Amin et al., 2007; Lyhs et al., 2010; O'Leary et al., 2011). However, the use of multiple phenotypic and genotypic typing methods improves bacterial species and subspecies discrimination and analysis of *Campylobacter jejuni* and *Campylobacter coli* heterogeneity (Wassenaar and Newell, 2000).

The aim of the present study was to perform a comprehensive molecular characterization of *Campylobacter* isolated from cattle during the slaughter process, basing on the identification of 11 different putative and toxin genes. Moreover, the strains were genotyped with the PFGE method and their resistance to several antimicrobials was also investigated.

2. Materials and methods

2.1. Sample collection

A total of 406 cattle slaughtered during November 2007–June 2009 in 3 slaughterhouses in the eastern part of Poland were used in the study. The samples were collected as described previously (Wieczorek and Osek, 2010). Briefly, the bovine hides and the corresponding carcasses were surface swabbed at the brisket area using sterile sponges. To each swab, 200 ml of Maximum Recovery Dilution (MRD, Oxoid, United Kingdom) was added and stomached for 3 min. After centrifugation at 1000 × *g* for 15 min, pellets were re-suspended in 100 ml of selective enrichment Bolton broth plus 5% leaked horse blood and modified Bolton broth selective supplement (Oxoid) containing the following antimicrobials: vancomycin, cefoperazone, trimethoprim, and amphotericin B to prevent non-target microbials. The enrichment cultures were grown for 48 h at 41.5 °C under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2) and then plated onto Karmali agar (Oxoid) and *Campylobacter* blood free agar (Oxoid) with CCDA selective supplement (Oxoid) followed re-incubation under the previously described conditions for 48 h. Plates were examined for morphologically typical *Campylobacter* colonies (greyish, often with a metallic sheen, flat and moist with a tendency to spread). These colonies were confirmed by the microscopic morphology, motility, microaerobic growth at 25 °C and the presence of oxidase. The identified bacterial isolates were stored at −80 °C until further analysis.

2.2. PCR assays

2.2.1. Species identification

One bacterial isolate from each positive sample was tested using PCR. A bacterial colony was suspended in 1 ml of sterile water and centrifuged at 13,000 × *g* for 1 min. DNA was extracted using the Genomic-Mini kit (A&A Biotechnology, Poland) according to the manufacturer's instruction. *Campylobacter* species were identified using multiplex PCR (m-PCR) with three sets of primers specific for the simultaneous detection of the *C. jejuni* (the *mapA* gene target), *C. coli* (*ceuE* gene), and *Campylobacter*-specific *16S rRNA* gene as described previously (Wieczorek and Osek, 2005). Furthermore, in case of doubtful results, the second m-PCR was applied to identify the species-specific *hipO* and *23S rRNA* (*C. jejuni*), *glyA* (*C. coli*, *Campylobacter lari*, and *Campylobacter upsaliensis*), and *sapB2* (*Campylobacter fetus* subsp. *fetus*), respectively (Wang et al., 2002).

2.2.2. Detection of virulence genes

Campylobacter isolates were tested for the presence of the most often described virulence genes: *flaA*, *flhA*, *cadF*, *docA*, *cdtA*, *cdtB*, *cdtC*, *ciaB*, *iam*, *wlaN*, and *virB11*. The PCR conditions for all genes have been exactly the same as previously described (Wieczorek, 2010).

2.3. Antimicrobial susceptibility

A microbroth dilution method was used to establish the minimum inhibitory concentrations (MICs) of *Campylobacter* isolates to 7 antimicrobial agents using the Sensititre® custom susceptibility plates, EUCAMP (Trek Diagnostics, United Kingdom). Antimicrobials, dilution ranges, and cut-off values used for MIC determination are described in Table 1. The strains were subcultured twice on Columbia agar (Oxoid) at 41.5 °C for 48 h under microaerobic conditions. The minimum inhibitory concentration of the antimicrobial agents was determined using Mueller–Hinton Broth (Oxoid) supplemented with 2–2.5% horse blood (Trek). The plates were incubated at 37 °C for 48 h under microaerophilic conditions and read using the Vision® system (Trek). The antimicrobials and cut off values used for the interpretation of the MIC results were in accordance with EUCAST (www.eucast.org) and the European Union Reference Laboratory for Antimicrobial Resistance.

2.4. PFGE analysis

All *Campylobacter* isolates were typed by pulsed field gel electrophoresis (PFGE) using the standard operating procedure of PulseNet (Ribot et al., 2001). In summary, the plugs were prepared from 400 μl of bacterial suspensions to which 20 μl proteinase K (20 mg/ml) (Sigma Life Science, USA) and 400 μl of Seakem Gold Agarose (Lonza, USA) in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) were added. Bacterial cell lysis was performed with 50 mM Tris, 50 mM EDTA, pH 8.0 + 1% Sarcosyl and 0.1 mg proteinase K per ml. The DNA was digested with *Sma*I enzyme (Fermentas, EU), 40 U at 25 °C for 4 h. *Salmonella* Braenderup H9812 was used as the molecular weight size standard. PFGE was performed using the CHEF DR II System (Bio-Rad, USA) with the following parameters: initial switch time of 6.8 s, final switch time of 35.4 s for 18 h at 6 V and 14 °C in 0.5 × TBE buffer (Sigma). The gels were stained with ethidium bromide (5 μg/ml) for 15–20 min, and the DNA banding pattern was captured with the Gel Doc 2000 system (Bio-Rad).

2.5. Reference strains

The following reference strains were included in the study: *C. jejuni* ATCC 33560, *C. coli* ATCC 43478, and *S.* Braenderup H9812 ATCC BAA-664.

Table 1
Antimicrobials, dilution ranges and cut-off values used for MIC determination of *Campylobacter*.

Antimicrobial class	Antimicrobials	Dilution range (mg/L)	Cut off values (mg/L)	
			C. jejuni	*C. coli*
Aminoglycosides	Gentamicin (GEN)	0.12–16	1	2
	Streptomycin (STR)	1–16	2	4
Macrolides	Erythromycin (ERY)	0.5–32	4	16
Quinolones and Fluoroquinolones	Nalidixic acid (NAL)	2–64	16	32
	Ciprofloxacin (CIP)	0.06–4	1	1
Tetracyclines	Tetracycline (TET)	0.25–16	2	2
Amphenicols	Chloramphenicol (CHL)	2–32	16	16

Download English Version:

https://daneshyari.com/en/article/6288845

Download Persian Version:

https://daneshyari.com/article/6288845

Daneshyari.com