

Original article

MINIMUM INHIBITORY CONCENTRATION AND MINIMUM BACTERICIDAL CONCENTRATION OF CEFTIOFUR AMONG *CAMPYLOBACTER JEJUNI* SEROTYPES ISOLATED FROM RETAIL CHICKEN CUTS IN KHON KAEN PROVINCE OF THAILAND

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Abstract The aim of this study was to assess the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 54 *Campylobacter jejuni* isolates obtained from retail chicken cuts during December 2007 to May 2008 in Khon Kaen Province of Thailand using broth microdilution method. *C. jejuni* were tested on the cation adjusted CaCl₂ and MgCl₂ Muller Hinton Broth (CAMHB) with 5% (v/v) defibrinated. The antimicrobial susceptibility test revealed an outstanding percentage of 98.15% (53 out of 54) resistance to ceftiofur (CTF). The minimum inhibitory concentration (MIC) breakpoint for resistance of CTF was ≥ 8 $\mu\text{g/ml}$. Among the 54 *C. jejuni* tested, the MIC of > 256 (3 isolates), 256 (29 isolates), 128 (18 isolates), 64 (2 isolates), 32 (1 isolate), and 2 $\mu\text{g/ml}$ (1 isolate) was evident. Only 1 isolate of serotype A was susceptible to CTF. Also, 29 and 50 out of 54 isolates had the MIC and MBC of 256 $\mu\text{g/ml}$. Serotype A (20.37%, 11 out of 54) was the most frequently found serogroup of *C. jejuni* resistant to CTF. No serotyping discrepancy was found for the resistant profile of this bacterium to CTF. Strict concern needs to be done in an attempt to use CTF for veterinary medicine. **Chiang Mai Veterinary Journal 2009;7(2):107-113.**

Keywords: broth microdilution method, *Campylobacter jejuni*, ceftiofur (CTF), MBC, MIC

Introduction

Ceftiofur (CTF) is the third generation cephalosporin antimicrobial, it has been used in veterinary medicine. Ceftiofur sodium was administered to breeding cattle and lactating cattle with foot rot or papillomatous digital

dermatitis (PDD). Previous study⁽¹⁾ noted that 1 g or 2 g of CTF was administered intramuscularly in breeding and lactating cows, respectively. As a result, the clinical manifestation was drastically improved in breeding and lactating cows. CTF is

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resistant to the antibiotic resistance enzyme beta-lactamase, and has activity against gram-positive and gram-negative bacteria.

Thermophilic bacteria of the genus *Campylobacter* spp. are foodborne enteric pathogens, and *C. jejuni* is the most commonly reported cause of gastroenteritis in humans ⁽²⁾. The association between *Campylobacter* in poultry and human enteritis is due to the persistence of this agent in the rearing environment of broilers, which asymptotically colonizes their intestine and eventually contaminates the carcasses ⁽³⁾. The development of antibiotic resistance in many countries, mainly among zoonotic microorganisms and the impact of foodborne diseases on consumers may be devastating to the food industry ⁽⁴⁾. The increase in the antimicrobial resistance of *Campylobacter* may lead to treatment failure in severely affected humans ⁽⁵⁾. Many factors, such as host susceptibility, virulence of the infecting strain and fat content of the meat, influence the pathogenesis of *C. jejuni*. Surprisingly, the elderly are not particularly predisposed to *C. jejuni* illness but children under five years of age, young adults aged 20–25, and immunocompromised individuals are at greatest risk of *C. jejuni* illness. The increasing of antimicrobial resistance in *C. jejuni* is particularly alarming since resistant organisms may be vertically

transmitted through the food supply to humans and, hence, may represent a significant public health threat. In Thailand, limited information was found concerning CTF. This study aimed at investigating the CTF MIC/MBC for *C. jejuni* isolated from Thai chicken cuts available to retail sale outlets in Khon Kaen province. Also, different in serotypes of *C. jejuni* were of interest as a factor that may contribute to the susceptibility or resistance of this bacterium. Therefore, the aim of this study was to assess the in vitro antimicrobial resistance of *C. jejuni* strains isolated from chicken cuts, focusing on CTF, a cephalosporin approved for veterinary uses and also, major serotypes that may contribute to the different in antimicrobial profiles of *C. jejuni* isolates to CTF.

Materials and methods

Isolation of *Campylobacter* spp. from chicken cuts: Chicken cut samples were collected from superstores, in Khon Kaen province from December, 2007 to May, 2008. Samples were examined for contamination of *Campylobacter* species according to the standard conventional plating procedure ^(6 - 8). Briefly, isolation of the campylobacters was done by direct inoculation on *Campylobacter* Blood-Free Selective Agar (modified CCDA, Oxoid, UK). After samples were macerating for 2 min, then 2 loopfuls of macerating fluid was streaked onto mCCDA Agar (modified CCDA- Preston) (CM0739, Oxoid, UK)

with CCDA Selective Supplement (SR0155E, Oxoid, UK), and *Campylobacter* Growth Supplements, SR 0232E (Oxoid, UK) for isolated colonies. Incubation was done at 42°C for 48 hr in microaerophilic atmosphere generated by Anaerocult[®]C (Merck, Germany). Well isolated clones were picked up and examined biochemically for catalase, oxidase, and s-shaped or gull-wing shaped morphology for genus.

Hip-O PCR screening for *C. jejuni* : Identification of *C. jejuni* was confirmed by PCR using *hip-O* (725 bp, Takara, Japan) for *C. jejuni*⁽⁶⁾. Briefly, master mix for x1 µl reaction, was DW, 37.50 µl; 10x PCR buffer minus Mg²⁺ 5 µl, 10 mM dNTP mixture 4 µl; Primer F (10 µM each) 0.50 µl; Primer R 0.50 µl; ExTaq, 0.50 µl; DNA template, 2 µl; total volume for PCR mixture was 50 µl. PCR conditions were as followed: 94°C, 2 min; 94°C, 30s; 66°C, 30s; 72°C, 60s; 72°C, 7 min; 4°C, ∞ for 35 cycles.

Serogrouping method : They were 25 serotypes of *C. jejuni* by Penner's method i.e. A, B, C, D, E, F, G, I, J, K, L, N, O, P, R, S, U, V, Y, Z, Z₂, Z₄, Z₅, Z₆, Z₇, respectively^(6, 9). One drop of *Campylobacter* antisera was used followed the manufacturer's instruction (Denka Ltd., Tokyo, Japan) into the microtiter plates containing activated chicken red blood cells coated with antigen of *C. jejuni*. Microplates were incubated for 30 min in moist chambers before assessing the result.

Antimicrobial susceptibility test : Broth microdilution method was employed in this study followed the procedure indicated in the CLSI guidelines. Mueller-Hinton Broth, CAMHB, supplemented with 5% (v/v) defibrinated sheep blood was used as a medium of choice. Freshly grown isolates were prepared in 0.85% (w/v) physiological saline and equilibrated to 0.5 McFarland standards. Then CTF solutions were allotted into third well (96-wells sterile microtiter plates) and twofold serially diluted until the last tested concentrations (256 - 0.06 µg/ml). Then cell suspension was applied into each well, mixed, and incubated at 42°C for 48 hr in the microaerophilic atmosphere generated by Anaerocult[®]C (Merck, Germany). Interpretation of MIC for CTF was made compared to the cut off value of ≥ 8 for resistance.

Results

Among the 54 *Campylobacter* isolates tested, the MIC of > 256 µg/ml (3 isolates), 256 (29 isolates), 128 (18 isolates), 64 (2 isolates), 32 (1 isolate), and 2 (1 isolate) was evident. Only 1 isolate was susceptible to CTF. The remainder was all resistant to this drug. Among the isolates tested, majority of them were categorized into serotype A (20.37%, 11 out of 54 isolates) while serotype L and Y ranked second (11.11%, 6 out of 54 isolates, each). Multiple serotypes were merely found in 2 isolates (3.70%, 2 out of 54 isolates) as indicate in Figure 1.

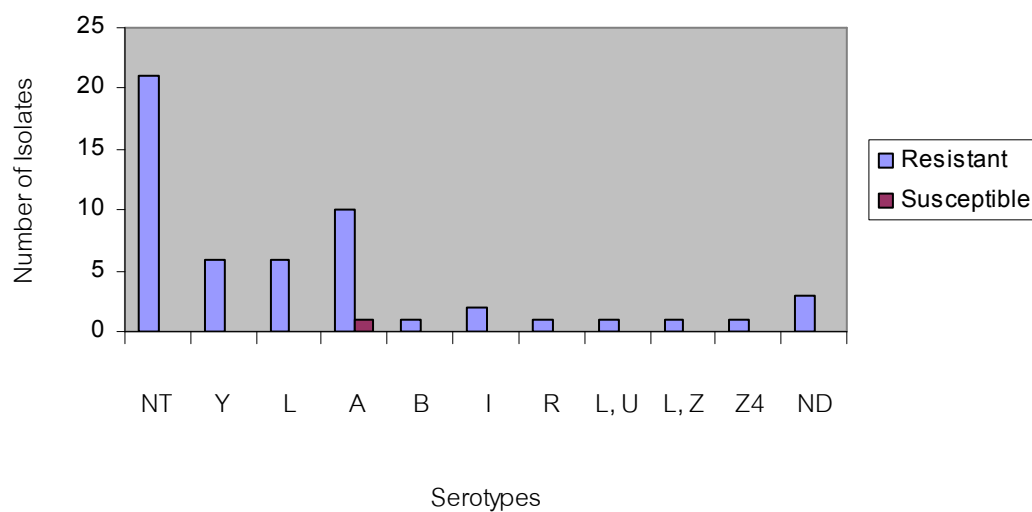


Figure 1. CTF antimicrobial profiles by serotypes of *C. jejuni* isolated from chicken cuts

MIC breakpoints for resistant of CTF was ≥ 8 $\mu\text{g/ml}$, according to the interpretative standards established by the CLSI for bacteria isolated from animals. Table 1 shows the MIC and MBC of

C. jejuni for CTF. MIC and MBC of 256 $\mu\text{g/ml}$ was the most frequently found i.e. 29 and 50 out of 54 isolates, respectively.

Table 1. MIC and MBC of *C. jejuni* isolated from chicken cuts for CTF

Antimicrobial Concentrations ($\mu\text{g/ml}$)	% (No. of isolates/Total no. of isolates)	
	MIC	MBC
> 256	5.56 (3/54)	5.56 (3/54)
256	53.70 (29/54)	92.59 (50/54)
128	33.33 (18/54)	1.85 (1/54)
64	3.70 (2/54)	0 (0/54)
32	1.85 (1/54)	0 (0/54)
2	1.85 (1/54)	0 (0/54)
Total	100 (54/54)	100 (54/54)

Discussions

Use of antimicrobials in livestock productions is controversial and may lead to the emergence of resistant organisms that could be transmitted to humans through the food supply. High levels of resistance in food *C. jejuni* isolates were observed for CTF (58%). Nevertheless, Kuana et al.⁽¹⁰⁾ noted a 33% resistance of *Campylobacter* species to CTF. Sahin et al.⁽¹¹⁾ stated that *Campylobacter* species are intrinsically resistant to cephalosporin, including CTF, and these compounds often are included in the selective medium for the isolation of *Campylobacter*⁽¹²⁾. Regardless of the inclusion of CTF in the selective media, it is frequently used in food animal production for the treatment of diseases caused by other bacterial pathogens. Strikingly, 100% of the tested *C. jejuni* isolates were resistant to this drug⁽¹¹⁾, while this study encountered a slightly lower i.e. 98.15% resistant to CTF. Precaution has to be taken when attempting to use CTF in veterinary medicine since resistant rate was high (98.15%) in this study as well as in previous studies (33, 58, and 100%). Likewise, when sub-typing *C. jejuni*, it was evident that serotype A dominated (20.37%, 11 out of 54) in chicken cut samples collected from northeastern Thailand. Similar finding was noted (27%, 8 out of 29) in chicken faecal isolates from the north⁽¹³⁾.

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