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In vitro susceptibility of *Campylobacter jejuni* from Kuwait to tigecycline & other antimicrobial agents

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***Background & objectives*: There is an increasing frequency of resistance of *Campylobacter jejuni* to antimicrobial agents making treatment difficult. In this study, the *in vitro* susceptibility of *C. jejuni* isolates collected over an eight year period was tested against tigecycline, a glycylcycline, the previously tested antimicrobial agents in Kuwait, ciprofloxacin, erythromycin and tetracycline, and other antimicrobial agents not previously tested in Kuwait, amoxicillin-clavulanic acid, gentamicin, imipenem and meropenem.**

***Method*s: A total of 97 *C. jejuni* isolates from diarrhoeal stools of Kuwaiti patients during 2002-2010 were studied for susceptibility to the above antimicrobial agents by E test.**

***Results*: Erythromycin resistance increased from 5.0 per cent in 2002-2003 to 13.8 per cent in 2007-2010. The figures for ciprofloxacin resistance for the same periods were 53 and 65.5 per cent, respectively. Tetracycline resistance increased from 40.0 per cent in 2003-2006 to 62.1 per cent in 2007-2010 (*P*=0.05). However, all isolates were uniformly susceptible to tigecycline and other antimicrobial agents.**

***Interpretation & conclusions*: There was a progressive increase in the prevalence of resistance to ciprofloxacin, erythromycin and tetracycline. As all isolates were uniformly susceptible to tigecycline, this antimicrobial agent can be considered as a potential candidate for treatment in clinical studies.**

Key words Antibiotic susceptibility - antimicrobial agents - *C. jejuni* - tigecycline

Although most *Campylobacter jejuni* infections are self-limiting and do not require treatment, antimicrobial therapy is required for severe infections[1]. Increasing resistance of *C. jejuni* to antimicrobial agents has been reported from various parts of the world[2]. Tigecycline is a member of a new group of antibiotics, the glycylcyclines, with potent activity against a broad spectrum of bacteria. It could be an alternative drug for treatment of *C. jejuni* infections. There are only a few reports available in literature on *in vitro* susceptibility of *Campylobacter* spp. to tigecycline[3-5]. There could be geographical variation in the susceptibility of isolates. Therefore, *C. jejuni* isolates from patients with diarrhoea in Kuwait were tested against this agent and other clinically relevant antimicrobial agents that have not been tested previously in Kuwait. These included amoxicillin-clavulanic acid, gentamicin, imipenem and meropenem. It was also studied whether the prevalence

of resistance to previously tested agents in Kuwait *viz*. erythromycin, ciprofloxacin and tetracycline[1,6] has increased in recent times.

A recommended method for *in vitro* susceptibility testing of *C. jejuni* to antimicrobial agents by Clinical and Laboratory Standards Institute (CLSI), USA, is agar dilution[7]. A significant correlation has been shown between E test and agar dilution for a number of antimicrobial agents[8]. Since E test has been used to study tigecycline susceptibility[4], we used this test in our study.

Material & Methods

Bacterial isolates: *C. jejuni* isolates were cultured from the stools of patients treated for diarrhoea at the Mubarak Al-Kabir Hospital affiliated to Kuwait University, Kuwait, during 2002-2010. There were 25 isolates from 2002-2003, 43 isolates from 2004-2006 and 29 isolates from 2007-2010 periods. In a previous study on *in vitro* susceptibility to ciprofloxacin and erythromycin, 50 consecutive *C. jejuni* isolates during 2000-2003 were tested[1]. In the current study, 25 of these isolates (representing every other isolates) were included from 2002-2003. Similarly, in a previous study on *in vitro* susceptibility to tetracycline, 85 consecutive isolates obtained during 2003-2006 were tested[6]. In the current study, 43 of these (representing every other isolates) were included from 2004-2006. The remaining 29 isolates (these included all isolates) were from 2007-2010 period (the number of isolates available during these years was small because of decreased isolation during these years). All 97 isolates were tested for susceptibility to amoxicillin-clavulanic acid, gentamicin, imipenem, meropenem, and tigecycline. In addition, the 43 isolates from 2004-2006 (which were not previously tested for susceptibility to ciprofloxacin and erythromycin) and all the 29 isolates from 2007-2010 period were tested for susceptibility to ciprofloxacin and erythromycin. The 29 isolates from 2007-2010 were also tested for susceptibility to tetracycline. A local *C. jejuni* strain 68, susceptible to all antimicrobial agents tested, was used as a control.

The minimum inhibitory concentrations (MICs) for antimicrobial agents were determined by the E test (AB Biodisk, Solna, Sweden) using Mueller-Hinton agar and incubating the culture in a microaerobic atmosphere at 37°C for 48 h[1]. The MIC breakpoints for resistance for the antimicrobial agents, amoxicillin-clavulanic acid, ciprofloxacin, erythromycin, tetracycline, gentamicin, imipenem, meropenem and tigecycline were as recommended by Lehtopolku *et al*[4] (Table).

The sample size was computed to detect clinically important differences in the prevalence of resistance between different time periods assuming a change in the resistance prevalence of >30 per cent to be clinically important. A sample size of 25 was required to detect a difference of 30 per cent in resistance. The significance of differences in proportions of resistance isolates was calculated by Chi square test. $P \leq 0.05$ was considered significant.

Results & Discussion

For comparison of resistance of isolates to erythromycin, ciprofloxacin and tetracycline of our previous two studies[1,6] with that of the more recent isolates, the isolates were grouped into three study periods (Table). All the isolates were uniformly susceptible to tigecycline, gentamicin, imipenem, meropenem and amoxicillin-clavulanic acid. The resistance to ciprofloxacin in period I was 53 per cent (34/64 of isolates resistant)[1]. This resistance rate gradually increased in periods II and III with no statistically significant differences in the increases. The resistance rate of the isolates to erythromycin was 5 per cent (3/64 of the isolates resistant) in period I[1]. This rate decreased in period II and then increased in period III with no statistically significant differences. The resistance to tetracycline was 40 per cent (34/85 of the isolates resistant) among the 2003-2006 isolates[6]. This resistance significantly increased to 62.1 per cent among the 2007-2010 (period III) isolates (P=0.05).

Among the total isolates, 37 were resistant to two antimicrobial agents (ciprofloxacin + tetracycline, 35; ciprofloxacin + erythromycin, 2) and four were multi drug-resistant (ciprofloxacin + tetracycline + erythromycin). However, the MICs of tigecycline for these resistant isolates were similar to those of the susceptible isolates as the values for both categories varied between <0.016 to 0.094 µg/ml (data not shown).

Our data on tigecycline susceptibility are similar to those of previous studies where all *Campylobacter* spp. isolates were susceptible to tigecycline[3-5]. As reported by other studies[4,9], our isolates were susceptible to the carbapenems, meropenem and imipenem and gentamicin. High levels of *in vitro* activity of amoxicillin-clavulanic acid against *Campylobacter* spp. have also been reported in another study[9]. The prevalence of resistance to ciprofloxacin

Table. Susceptibility of *C. jejuni* isolated in different years to various antimicrobial agents

Antimicrobial agent (resistant breakpoint, µg/ml)[a]	Years (n= no. of isolates)			
	2002-2003 (Period I) (n=25)	2004-2006 (Period II) (n=43)	2007-2010 (Period III) (n=29)	Total (n=97)
	MIC_{90} (range) No.(%) resistant	MIC_{90} (range) No. (%) resistant	MIC_{90} (range) No. (%) resistant	MIC_{90} (range) No. (%) resistant
Amoxicillin-clavulanic acid (≥32)	1 (0.25-3) 0 (0)	1 (0.019 -3) 0 (0)	2 (0.094 -4) 0 (0)	1.5 (0.019 -4) 0 (0)
Ciprofloxacin (≥4)	NT[b]	>32 (0.023 ->32) 26 (60.4)	>32 (0.046 ->32) 19 (65.5)	>32 (0.023 ->32) 45 (62.5)[c]
Erythromycin (≥16)	NT	1 (0.064 ->256) 1 (2.3)	>256 (0.019 ->256) 4 (13.8)	2 (0.016 ->256) 5 (6.9)[c]
Tetracycline (≥16)	NT	NT	>256 (0.032 ->256) 18 (62.1)	>256 (0.032 ->256) 18 (62.1)[d]
Gentamicin (≥16)	1 (0.25-6) 0 (0)	1.5 (<0.064 -2) 0 (0)	1.5 (0.05 -1.5) 0 (0)	1.5 (<0.064 -6) 0 (0)
Imipenem (≥16)	0.064 (<0.002-0.125) 0 (0)	0.094 (0.008 -0.125) 0 (0)	0.0125 (0.003 -0.32) 0 (0)	0.094 (<0.002 -0.32) 0 (0)
Meropenem (≥16)	0.016 (0.008-0.064) 0 (0)	0.032 (0.003 -0.125) 0 (0)	0.047 (0.002 -0.047) 0 (0)	0.032 (0.002 -0.125) 0 (0)
Tigecycline (≥0.5)	0.023 (<0.016 -0.094) 0 (0)	0.016 (<0.016 -0.047) 0 (0)	0.032 (0.016 -0.094) 0 (0)	0.032 (<0.016 -0.094) 0 (0)

[a]Breakpoint values as per recommendation[4] ; NT[b], Not tested in the current study, but tested in the previous studies[1,6] with a different number of isolates.
[c]The denominator for percentage calculation is 72 isolates; [d]the denominator for percentage calculation is 29 isolates; for all others the denominator is 97 isolates.
For ciprofloxacin, the *P* values for differences between periods I&II, and between periods I & III are 0.56 and 0.37, respectively; for erythromycin, the corresponding values are 0.20 for both.
For tetracycline, the *P* value for difference between 2003-2006 isolates [40% (34/85 resistant)][6] and period III isolates is 0.05

and erythromycin gradually increased over the three study periods even though the differences were not statistically significant. However, the increase in resistance rate to tetracycline compared to the 2003-2006 period[6] was statistically significant.

Based on the *in vitro* susceptibility, orally administered antimicrobials useful for treatment of *C. jejuni* diarrhoea are erythromycin and amoxicillin-clavulanic acid. Although, the organisms were uniformly susceptible to other agents, these antimicrobials are useful for treatment of extra-intestinal infections only being parenterally administered antimicrobials. Tigecycline, another parenterally-administered antimicrobial has shown an excellent activity in the treatment of extraintestinal campylobacteriosis. The major route of elimination of tigecycline is through the faeces[10]. Therefore, it seems reasonable to assume that tigecycline might be effective even for patients with diarrhoea. The MICs of tigecycline for multidrug-resistant isolates and for susceptible isolates were similar in our study as reported in other studies[3,4]. This is a welcome finding for potential treatment of multidrug-resistant *C. jejuni* infections with tigecycline.

In conclusion, there was an increase in the prevalence of resistance to erythromycin, ciprofloxacin and tetracycline in *C. jejuni* isolates of recent years in Kuwait, limiting their usefulness in treatment. As all *C. jejuni* isolates were uniformly susceptible to tigecycline, it can be considered as a potential alternative drug in clinical studies for treatment of *C. jejuni* infections.

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