High level of ciprofloxacin resistance and its molecular background among *Campylobacter jejuni* strains isolated in the United Arab Emirates

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The antibiotic sensitivity and the serotype and molecular type (MT) distribution of 41 *Campylobacter jejuni* strains isolated from individual patients in Tawam Hospital, Al Ain, United Arab Emirates, were investigated. While all strains were sensitive to erythromycin (MIC 0.5–4 mg l⁻¹), 35 isolates (85.4 %) exhibited resistance to ciprofloxacin (MIC 8–64 mg l⁻¹). All resistant strains carried the Thr-86 to Ile mutation in the gyrase A (*gyrA*) gene, as shown by mismatch amplification mutation assay (MAMA) and confirmed by sequencing. Based on the partial sequences of *gyrA*, resistant isolates carried 10 distinct alleles, eight of them representing new variants. Strains were assigned to 30 MTs based on the combined results of PFGE and *flaA* PCR-RFLP typing. Eight of the 35 ciprofloxacin-resistant strains, isolated over a period of more than 1 year, represented the largest MT, also carrying the same allelic variant of the *gyrA* gene. These results show that the local incidence of fluoroquinolone resistance among *C. jejuni* is one of the highest reported worldwide. It was also demonstrated that stable MTs could persist for a relatively long time among the clonally unrelated antibiotic-resistant isolates of *C. jejuni*. The data also emphasize the need to replace fluoroquinolones as empirical therapy for diarrhoea of undiagnosed aetiology.

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INTRODUCTION

Fluoroquinolone resistance in *Campylobacter jejuni*, a leading cause of gastrointestinal infections worldwide and one of the most important causes of travellers' diarrhoea, is rapidly increasing. Resistance is commonly due to point mutations in the quinolone resistance determinant region (QRDR) of the gyrase A (*gyrA*) gene involving the Thr-86 for high, and the Asp-90 and Ala-70 amino acid residues for lower-level resistance (Engberg *et al.*, 2001).

While *C. jejuni* infections usually present as acute self-limiting gastrointestinal illnesses, in severe or prolonged

cases antibiotic therapy is indicated, with macrolides as the primary drug of choice (Butzler, 2004). However, particularly if the causative agent is not identified, as is often the case in travellers' diarrhoea, fluoroquinolones are frequently used empirically (Guerrant *et al.*, 2001; Yates, 2005). In such cases, resistance to ciprofloxacin can result in therapeutic failure (Guerrant *et al.*, 2001; Sanders *et al.*, 2002). Moreover, by mechanisms yet to be elucidated, fluoroquinolone resistance appears to coincide with increased fitness of the organism. In humans, fluoroquinolone-resistant strains are shed longer than sensitive isolates (Nelson *et al.*, 2004; Engberg *et al.*, 2004), and they have been reported to out-compete their sensitive counterparts, at least in certain genetic backgrounds, when colonizing poultry, i.e. the natural reservoir of the pathogen (Luo *et al.*, 2005). All these findings underscore the necessity for close monitoring of the incidence and spread of fluoroquinolone-resistant clones of *C. jejuni*.

Countries on the Arabian Peninsula, some of them increasingly popular tourist destinations, have recently

Abbreviations: MAMA, mismatch amplification mutation assay; MT, molecular type; QRDR, quinolone resistance determinant region; UAE, United Arab Emirates.

The GenBank/EMBL/DDBJ accession numbers for the partial sequences of the *gyrA* genes representing new allelic variants are DQ449657, DQ449658, DQ449659, DQ449660, DQ449661, DQ449662, DQ449663 and DQ449664.

been scored as areas of high risk to acquire campylobacter infections (Ekdahl & Andersson, 2004). However, data on the incidence of fluoroquinolone resistance and on the type of strains present in the region are very limited (Sjogren *et al.*, 1989; Albert *et al.*, 2005; Jumaa & Neringer, 2005). The aim of the current study was to investigate the variety of *C. jejuni* strains isolated between 2002 and 2004 in a tertiary care hospital in Abu Dhabi Emirate, United Arab Emirates (UAE), and to determine the frequency and the genetic background of fluoroquinolone resistance among the local isolates.

METHODS

Strains and culture conditions. Forty-one *C. jejuni* strains isolated from faecal samples of individual patients in the Microbiology Laboratory of Tawam Hospital, Eastern Region of the Abu Dhabi Emirate, UAE, between September 2002 and September 2004 were studied. Tawam Hospital is a tertiary care teaching hospital, one of the two major hospitals in the city of Al Ain, which has a population of approximately 350 000. The 41 strains represented 63 % of the 65 *Campylobacter* isolates recovered from the 3389 stool samples tested during this period of time. Seven of these strains were not available for us due to technical reasons, six represented repeated isolates and 11 were species other than *C. jejuni*. Four of the 41 *C. jejuni* strains finally included in the study were recovered from inpatients. No detailed data (except the diagnosis 'diarrhoea' or 'gastroenteritis') on the medical conditions, therapeutic or travel history of the patients were available to us. Strains were stored in tryptic soy broth (Oxoid) containing 10 % (v/v) glycerol at -80°C , and were routinely cultured on campylobacter agar base (Oxoid) supplemented with 5 % (v/v) sheep blood, under microaerophilic conditions generated with CampyGen (Oxoid) bags in jars at 36°C for 48 h. The identification of the isolates was by colonial morphology and Gram-stain characteristics, and confirmed by the API Campy kit (bioMérieux) according to the manufacturer's instructions.

Antibiotic susceptibility testing. The MICs of nalidixic acid, ciprofloxacin and erythromycin were determined by the agar dilution method using Mueller–Hinton agar (Oxoid) supplemented with 5 % (v/v) sheep blood and incubated under microaerophilic conditions at 36°C for 48 h. The final antibiotic concentrations in the agar plates ranged from 0.25 to 512 mg l^{-1} , according to the standards of the Clinical and Laboratory Standards Institute (CLSI; formerly known as the National Committee for Clinical Laboratory Standards, NCCLS) (NCCLS, 2003, 2005). Reference strain *C. jejuni* ATCC 33560 was included in each run as control.

Serotyping. Serotyping, based on the heat-stable antigen of the strains, was carried out by passive haemagglutination (Penner & Hennessy, 1980) using a set of commercial antisera (Denka Seiken) according to the manufacturer's instructions.

DNA purification. Genomic DNA was purified with a QIAamp DNA Mini kit (Qiagen) according to the manufacturer's instructions.

Mismatch amplification mutation assay (MAMA) PCR and sequencing of the QRDR of gyrase A. The MAMA PCR detection of the Thr-86 to Ile mutation in the *gyrA* gene of the quinolone-resistant isolates was carried out according to the method of Zirnstein *et al.* (1999). A 673-bp-long part of the *gyrA* gene, containing the QRDR, was amplified using primers GZgyrA5 and GZgyrA6 (Zirnstein *et al.*, 1999), and directly sequenced by fluorescence-based direct sequencing employing the ABI Prism Dye Terminator cycle sequencing ready reaction kit (Perkin-Elmer) and an ABI Prism 310

genetic analyser automated sequencing system (Perkin-Elmer). For the analysis of the sequences, the program MEGA version 3.1 (Kumar *et al.*, 2004) was used.

PCR-RFLP. The amplification of the *flaA* gene using consensus primers, and the digestion of the amplicon with *DdeI* were carried out as described by Wassenaar & Newell (2000). The digested products were run in a 2 % agarose gel at 1 V cm^{-1} for 120 min. Photographs of gels stained with ethidium bromide were analysed by the GelCompare II software (Applied Maths).

PFGE. *C. jejuni* genomic DNA was digested with *SmaI* following the formaldehyde inactivation of DNase, and the fragments were subsequently separated on a CHEF DRII apparatus (Bio-Rad) as described by the 'Campynet' PFGE Subtyping Subgroup (2000). The macrorestriction patterns of the isolates were compared according to the Dice similarity index (1–1 % tolerance interval) using the GelCompare II software. A PFGE group was arbitrarily defined as a strain if it exhibited macrorestriction band patterns with at least 95 % similarity.

RESULTS AND DISCUSSION

Antibiotic susceptibility and serotype distribution of the isolates

All isolates were sensitive to erythromycin (MIC 0.5–4 mg l^{-1}), but 35 out of the 41 isolates (85.4 %) were resistant to nalidixic acid and ciprofloxacin with MIC values of between 128 and 512 mg l^{-1} , and 8 and 64 mg l^{-1} , respectively.

Although fluoroquinolone resistance in campylobacter is rapidly emerging all over the world, high incidences comparable to the one found in our study have only been reported from a few locations, such as 88 and 75 % in Spain (Ruiz *et al.*, 1998; Saenz *et al.*, 2000), 96 % in Thailand (Sanders *et al.*, 2002), 85.9 % in Hong Kong (Chu *et al.*, 2004), and 77.1 % in India (Jain *et al.*, 2005). Data from the Middle East are sparse. In 1998, Talhouk *et al.* (1998) reported a considerably lower level of ciprofloxacin resistance (39 %) in Lebanon among strains of human and animal origin. Recently, Albert *et al.* (2005) found 53 % ciprofloxacin resistance among human campylobacter isolates in Kuwait. It should be noted that the results of both the Kuwaiti study and our study were based on strains collected at single hospital laboratories. Further, more extensive, larger-scale studies should reveal whether or not the current studies reflect real differences in the incidences of fluoroquinolone resistance in this pathogen between the northern, and south-eastern parts of the Arabian Peninsula.

Earlier, Jumaa & Neringer (2005) had reported a 50 % fluoroquinolone-resistance rate among the *Campylobacter* spp. isolated from humans between 1999 and 2002, from the same hospital laboratory. The use of different methodologies in their study and the current study prevents the direct comparison of the results. However, the possibility of a considerable rise in fluoroquinolone resistance in the 2 years that followed their report cannot be excluded, and should be proven by further monitoring of its incidence.

A total of 31 (75.6%) of the 41 strains tested were serotypable, representing 10 serogroups. Serotype complex group HS4,13,16,43,50 was the most frequently encountered (22%), followed by serotype HS2 (14.6%); both are commonly encountered groups at other geographical locations also (Patton *et al.*, 1991; Nielsen *et al.*, 1997; Rautelin & Hänninen, 1999; Wareing *et al.*, 2002) (Fig. 1).

Molecular typing of the isolates

All strains were typable by both PFGE and PCR-RFLP. The 41 isolates belonged to 26 distinct PFGE groups (P1–P26); 21 of them consisting of single isolates only (Fig. 1). Two PFGE groups (P3 and P17) contained two, a further two types (P1 and P4) contained four, and a single type (P7) consisted of eight isolates. Of the 23 RFLP types (R1–R23), 15 were represented by single isolates only. Combining the results of the two typing methods, strains could be assigned to 30

distinct molecular types (MTs) (MT1–30). It is noteworthy that the eight isolates with identical PFGE patterns (P7) also exhibited identical RFLP patterns (R13) and formed a homogeneous MT (MT1) representing 19.5% of the total, and 22.8% of the ciprofloxacin-resistant strains (Fig. 1).

Similar to the results of others (Aarts *et al.*, 1995; Rautelin & Hänninen, 1999; Hänninen *et al.*, 2001), we found that the overlap between the results of sero- and molecular typing was not complete. Identical serogroups were encountered in different P and R groups (e.g. HS2 in P4, P11, P12, P14 and P15, or in R6, R8, R10, R16 and R17), while different serogroups could be detected within the same molecular groups (HS2 and HS1,44 in P4, or HS10 and HS1,44 and HS4,13,16,43,50 in R7). On the other hand, six of the eight strains in the cluster MT1 belonged to serogroup HS4,13,16,43,50, while two isolates were non-typable (Fig. 1). The six strains sensitive to fluoroquinolones

Fig. 1. MTs, sero- and allele types, antibiotic sensitivities and isolation dates of *C. jejuni* strains isolated in Al Ain, UAE. Abbreviations: NA, nalidixic acid; CIP, ciprofloxacin; NT, non-typable; ND, not done. Strains marked with the black bar represent members of a putative uniform clone.

Two further strains (#5171 and #102) carried the same allele of the *gyrA* gene as members of genotype group MT1, one of them belonged to serogroup complex HS4,13,16,43,50, while the other was non-typable (Fig. 1). However, these two isolates exhibited <90 % similarity by PFGE and <70 % similarity by PCR-RFLP to the other members of this group. The other large group of strains with an identical allele of the *gyrA* gene (G3) exhibited more extensive variations with the different typing techniques. They belonged to four different serogroups, and three isolates were non-typable. Furthermore, none of the isolates in this group exhibited similarities exceeding 85 % by PFGE and 80 % by PCR-RFLP to each other, as can be seen in Fig. 1.

Our data show a high incidence (85.4 %) of fluoroquinolone resistance among *C. jejuni* strains isolated in Al Ain, UAE. This observation is not biased by the fact that eight, i.e. 22.8 %, of the ciprofloxacin-resistant strains represented a single MT isolated over a considerable period of time. Even if members of MT1 had been counted as only one isolate, the incidence of fluoroquinolone resistance would still be 82.3 %, a very high figure by any comparison. This study, the first of this kind from the south-eastern part of the Arabian Peninsula, was too limited in scale to extrapolate the results to the whole UAE. Nevertheless, since some parts of the Middle East are increasingly frequented travel destinations, as well as relatively high-risk areas to acquire campylobacter infections (Ekdahl & Andersson, 2004), the data should stress the need for the establishment of an effective antibiotic surveillance in the region. Furthermore, they also indicate that alternatives to fluoroquinolones for the treatment of fairly severe diarrhoeal cases with unconfirmed aetiology should be sought.

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