Regional Review

Campylobacter enteritis in the Arabian Gulf

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Abstract

Diarrhoea illnesses constitute a common cause of morbidity and mortality worldwide. In recent years, *Campylobacter* spp. has been recognized as the leading cause of bacterial enteritis in both developed and developing countries. The biology of *Campylobacter*s as well as the mechanism by which they cause disease is yet to be fully explained. In addition, non-availability of fast and reliable diagnostic methodology and the growing trend of antibiotic resistance continue to pose significant challenges. The absence of national surveillance programs for campylobacteriosis, particularly in developing countries, makes it difficult to give an accurate picture of the true infection prevalence and the molecular epidemiology of isolates circulating in the populations, a situation which had hitherto existed in the Arabian Gulf region. However, in recent years, emerging data from studies in the Arabian Gulf region has not only enhanced our understanding of the epidemiology of *Campylobacter* both in humans and poultry in the region, but has also contributed to the overall understanding of the mechanism of *Campylobacter* enteritis, antibiotic resistance, and improved diagnostic approaches. In this review, we examine these emerging data from the Arabian Gulf region.

Key words: *Campylobacter*, virulence factors, antibiotic resistance, multiplex PCR

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Introduction

Diarrhoea illnesses constitute a common cause of morbidity and mortality worldwide. Several species of bacteria consumed through contaminated food or water cause bacterial enterocolitis and common culprits include *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., and *Escherichia coli* [1,2]. Although *Campylobacter jejuni* is now recognized as the leading bacterial cause of food-borne disease in both developed and developing countries [3-6], the biology of *Campylobacters* and the mechanism by which they cause disease is yet to be fully explained. In addition, non-availability of fast and reliable diagnostic methodology and the growing trend of antibiotic resistance are challenges which need to be addressed. In this article we examine emerging data on campylobacter infection from the Arabian Gulf region.

Biology

In 1866, the Austrian bacteriologist Theodor Escherich first described spiral bacteria in the colonic mucus of a child who died of *Cholera infantum* [7,8]. However, it was not until the early twentieth century that researchers were able to isolate the organism from animals using difficult and painstaking techniques [9]. Although about 16 species belonging to the genus *Campylobacter* have been identified, only two species, namely *Campylobacter jejuni* and *Campylobacter coli*, account for the majority of global infections. *Campylobacter* spp. are curved, S-shaped or spiral rods measuring 0.2-0.9 µm wide and 0.5-5.0 µm long. They are motile with a single, polar, unsheathed flagellum at one or both ends; however, some non-flagellated strains have been described. These Gram negative, non-spore forming, microaerophilic organisms tend to revert to non-culturable coccoid shapes under unfavorable conditions. They possess a small genome of approximately 1.6-1.7 Mbp with a GC ratio of about 30% and an AT ratio of 70%, and this small genome size perhaps explains their inability to ferment carbohydrates, their inability to degrade complex substances, and their requirement for special growth media [10]. Optimal culture conditions require temperatures ranging from 37ºC to 42ºC and a strict microaerophilic atmosphere (3–15% oxygen, 3–5% carbon dioxide and 85% nitrogen) [11].

Epidemiology

Campylobacter spp. reside in the gut of domesticated warm-blooded animals and birds as part of the intestinal microbiota [11]. Of particular importance to humans is their colonization of animals used in food production, including poultry, cattle, sheep and swine [5, 12]. *Campylobacter* enteritis is considered a food-borne zoonotic infection which is often acquired from a range of contaminated animal food products. The absence of national surveillance programs for *Campylobacter* infections, particularly in developing countries, makes it difficult to give an accurate picture of the true incidence for some populations, a situation which exists in the Gulf Co-operative Council (GCC) countries of the Arabian Gulf region. These GCC countries are Saudi Arabia, Bahrain, Kuwait, Oman, Qatar and United Arab Emirates. *Campylobacter* as a cause of diarrhoea illness in the Arabian Gulf region has been shown to range from 1.6-28% [1,13-15]. Earliest data reported from the Kingdom of Saudi Arabia in 1981 showed that *Campylobacter jejuni* accounted for 0.6% bacterial enteritis in a major referral center in Riyadh [16]. About 10 years later, another report from the same hospital showed that *Campylobacter* was isolated from the stool of 1% (82/7369) of children presenting with gastrointestinal symptoms [17]. A diversity in the occurrence of *Campylobacter* as an aetiological agent of bacterial enteritis is evident in reports from Saudi Arabia. Three studies reported in the 1990s have reported *Campylobacter* detection in patients with bacterial enteritis ranging from 2% [18], 4.5% [19] and 28% [13]. In Kuwait the reported data of *Campylobacter* enteritis was 2.2% in 1981and 7% in 1989 [15]. In the Kingdom of Bahrain, *Campylobacter* was detected in 1.6% of 426 children hospitalized with gastroenteritis between 1998 and 2002 [1]. This low level of detection might be due to the recent introduction of the isolation procedure in the reporting laboratory; hence only 426 of the 805 samples available in the study were tested for *C. jejuni*. In addition, the fact that only samples from hospitalized patients were assessed might also be a contributing factor. Indeed from 2002-2004, 96 cases of *Campylobacter* enteritis mainly in the paediatric age group, were identified in the same laboratory [20]. In all reports from the region, *Campylobacter* ranked among the top three aetiological agents of bacterial enteritis. In Jeddah, Saudi Arabia, *Campylobacter* infection was found to be second in prevalence to *Salmonella* with 69% of these infections being due to *C. jejuni* and 31% due to *C. coli* [19]. However, in data from Kuwait, *Campylobacter* ranked third after *Salmonella* and enterotoxigenic *E. coli* [21].

In developed countries, the incidence of infection is high among older children and young adults, with a male predominance [11,12] . Most cases are sporadic with occurrence of outbreaks in late summer and early fall. In contrast, *Campylobacter* infections occur more commonly in developing countries although clinical disease tends to be milder. In these areas, the infection is hyperendemic, and restricted to children; fewer cases are seen in adults as attenuation of symptoms with increasing age is the norm [12,22,23] Available data from the Arabian Gulf has shown significantly higher incidence among children, particularly those under the age of four years [15,17,20]. This data is similar to reports from other developing countries, such as Turkey and Egypt, where more episodes of *Campylobacter* enteritis per year in children under the age of three years have been observed [24,25]. In a recent report that describes *Campylobacter* infection in 96 patients in Bahrain, children under the age of three years were the most vulnerable population as 69% (66/96) of the patients were in this age group [20]. Few reports of *Campylobacter* infection in the neonatal age group have been documented, and this includes six cases of neonatal *Campylobacter* enterocolitis identified over a five-month period in a hospital in Riyadh, Saudi Arabia [26]. In addition, four cases of diarrhoea due to *Campylobacter lari* occurring in children under the age of three years has been reported from the region [27]. But it is also of interest that *Campylobacter* enteritis appears to also be an important cause of gastroenteritis in adults in this region, and in one study 36.5% of isolates were from adults aged 20 to 39 years [14,19].

In developing countries, *Campylobacter* enteritis has been described as being characterized by non-inflammatory, watery diarrhoea without blood, mucus or leucocytes. In addition, there is no strong pattern of seasonality as the infection is hyperendemic. However, although the infection occurs all year round in our region, definite peaks of infection in the winter months have been described [17,26]. Additionally, the frequent occurrence of bloody mucoid diarrhoea with leucocytes in the stool, which is similar to clinical presentations in developed countries, has been reported [17,18,21]. It has been suggested that the variation in the pattern of *Campylobacter* infection in developed versus developing countries is probably due to a higher rate

of exposure and infection early in life, resulting in different patterns of immunity [28]. It is interesting that the pattern seen in the Arabian Gulf appears to straddle those seen in developed and developing countries, and this could be due to the rapid economic changes which have occurred in the GCC countries in the past few decades.

Virulence factors and molecular characterization

The pathogenesis of *Campylobacter* enteritis is multifactorial and complex. Indeed, it has been said that the phenotypic traits associated with different strains may be related to their genetic diversity. The role of virulence factors such as motility, chemotaxis, colonization, adherence, invasion, translocation as well as toxin production have been studied and the mechanism(s) of pathogenicity is becoming clearer [5]. One of the top virulence candidates implicated in the pathogenesis of *Campylobacter* enteritis is toxin production, in particular cytolethal distending toxin (Cdt). This holotoxin exerts its toxigenic effect by damaging the DNA, which triggers cell cycle arrest and ultimate cell death [29]. However, the occurrence of inflammation, infiltration of the lamia propria by neutrophils, and bacteriaemia indicate that invasion is an important *Campylobacter* virulence determinant and it has been suggested that Cdt may also play a role in invasion. Other well-studied putative virulence genes associated with invasive capability are the invasion-associated marker (*iam*) and *Campylobacter* invasion antigens (*ciaB*) gene [30,31].

As the phenotypic traits associated with different strains may be related to their genetic diversity, we recently described the molecular characterization of *C. jejuni* isolates from Bahrain, and presented data which showed the relationship between the presence of combinations of these putative virulence genes, the invasive phenotype, and the severity of clinical infection [20,32]. These reports were significant as they provided the first insight into the molecular characterization of virulence genes in *Campylobacter* strains from the Arabian Gulf region. The findings confirm that *C. jejuni* of diverse genetic make-up and pathogenic potential are circulating in the population. The strains positive for *cia*, *iam* and *cdtB* genes exhibited the most invasive capability and they were also isolated from patients with the most severe infections. The presence of a combination of two genes conferred a variable degree of invasiveness with correlation between the presence of *ciaB* gene, the relative invasive potential, and clinical severity of infection. Interestingly, the absence of all three genes did not preclude *in vitro* invasiveness, indicative of a role for other still unidentified virulence genes. When the correlation between the presence of two virulence genes (*cdt* and *iam*) in *C. jejuni* and patients' demographic and clinical parameters was assessed, children under the age of three years were identified as the high risk group for *Campylobacter* enteritis [20]. Strains negative for both virulence genes ($cdtB^{-ve}/iam^{-ve}$) resulted in symptomatic infection in this age group, in sharp contrast with asymptomatic infection among older patients who were infected with these strains.

In recent years apoptosis induction and pro-inflammatory cytokine production during *C. jejuni* infection of the human monocytic cell line THP-1 have been reported. Indeed, apoptosis induction of host macrophages has emerged as a common virulence mechanism among enteric pathogens (*Salmonella*, *Shigella* and *Yersinia*). Similarly, emerging data from our group indicates that there is a correlation between the induction of apoptosis in THP1 cells and the number/type of virulence genes present in *C. jejuni* strains [33]. Indeed, the highly invasive cdt/iam/cia strains which we had previously described [32] induced the highest level of apoptosis.

Immune response

Although the immunopathogenic events in campylobacteriosis are yet to be fully understood, available data suggests that the immunological response plays a significant role in the pattern and mechanism of infection. This is consistent with reports showing a significantly higher level of serum antibodies to *C. jejuni* in Thai and Bangladeshi populations as compared to those in the USA [23,34,35].

It has been suggested that toxins produced by the bacteria modulate the immune response in both humans and animals. Elevation of proinflamatory cytokines and tumor necrosis factor alpha (TNF-α) have been been demonstrated in mice infected with *C. jejuni*. Using immunohistochemical techiques to detect intracellular production of cytokines and RT-PCR to evaluate for de-novo cytokine synthesis, the role of factors from living bacterial cells in directing the immune response during *Campylobacter* infection was first demonstrated in reports from Bahrain [36,37]. Exposure of INT407 cells to live bacteria, bacterial sonicates and bacterial filtrates showed that induction of interferon gamma, IL-10, TNF-α) and

IL-4 was significantly higher with the live bacterial cells. In addition, the preferential production of α and β chemokines, in particular IL-8, CCL2 and CCL4, were also demonstrated. These findings suggest that the innate and acquired immune response to *Campylobacter* infection recruits monocytes and CD4 lymphocytes. The immuopathogenic events possibly involve the release of IFN-γ and TNF-α, which induce the production of the chemokines (IL-8 & CCL2), which in turn activate monocytic cells with inflammatory consequences. The characterization of the factors from the living bacterial cells that direct this immune response has the potential for identification of immunotherapeutic candidates for use in *Campylobacter* infection.

There is an immunological basis to the development of GBS, which is perhaps the most notable complication of *Campylobacter* infection [38,39]. This condition manifests as a flaccid paralysis and has a significant association with serological evidence of recent infection with *Campylobacter* spp. In a 2002 report from Oman, 45.1% (37/82) of acute neuropathy in children identified over an eight-year period (1992-2002) were diagnosed as Guillain-Barré syndrome [40]. In Kuwait the overall annual incidence rate of 0.95/100,000 population at risk with a preponderance of cases in the winter months has been reported [41,42]. This association with the paediatric age group and winter peaks, which ties in with the regional epidemiological pattern of campylobacteriosis, suggests a strong causality for recent *Campylobacter* infection.

Campylobacter in poultry

Animals used in food production, particularly poultry, are now a recognized source of human campylobacter infection. Indeed, the ability of these bacteria to grow at 42^{o}C perhaps reflects their adaptation to the gut of some types of birds [9]. Broiler chicks become colonized at a very early stage in their lives and prevalence of colonization among poultry flocks can reach up to 100% in some areas [43]. From 2002 to2004, two studies were conducted to assess specimens obtained from slaughter-houses in Bahrain and Saudi Arabia for *Campylobacter* contamination. In one study, specimens consisting of 35 whole chickens, 27 chicken livers, and 38 chicken faeces were assessed using a combination of three culture methods, and just over half (57%) were found to be positive for *Campylobacter* contamination [44]. In another study, 60 chicken faeces specimens were assessed using a newly developed multiplex PCR technique with 100% *Campylobacter* detection [45]. However, in both studies, *C. jejuni* accounted for the majority of *Campylobacter* detected. The findings of this study, which is the first of its kind in our setting, indicates a need for increased surveillance and *Campylobacter* screening in food safety control to better protect consumers.

Antibiotic resistance

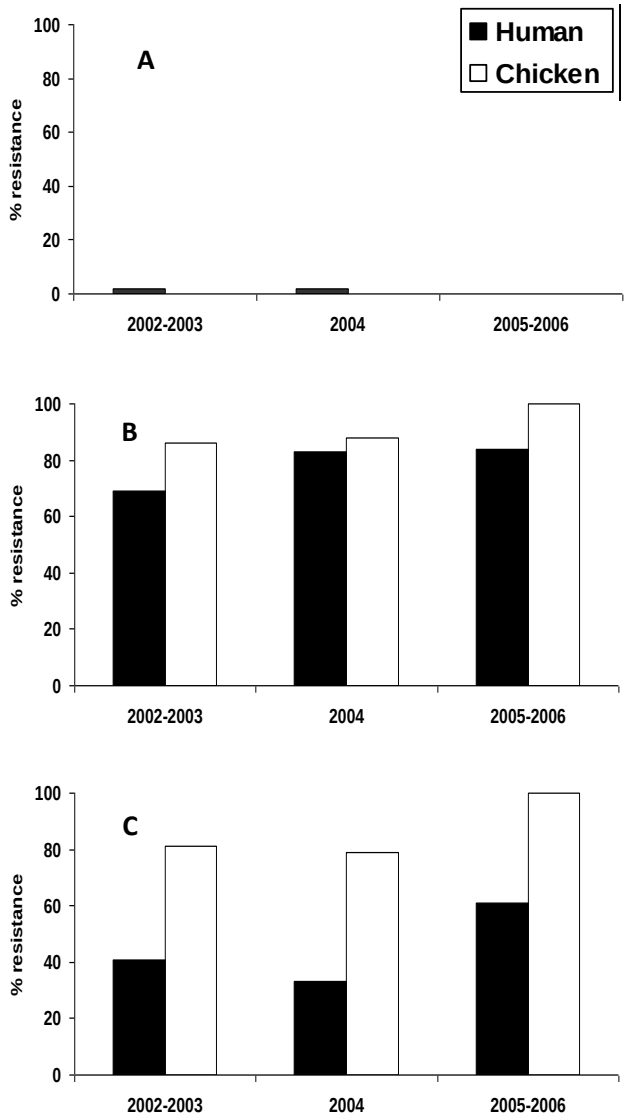
C. jejuni, which is the best characterized and most clinically relevant species in this genus, is sensitive to several classes of antibiotics, including macrolides (especially erythromycin), which have been traditionally utilized as first-line therapy, and quinolones such as ciprofloxacin [5]. While most cases of *Campylobacter* enteritis are self-limiting, in situations where antibiotic therapy is indicated either erythromycin or ciprofloxacin are the usual drugs of choice. However, recent data indicates an upward trend of *Campylobacter* resistance to antibiotics with varying patterns being seen in different countries and regions [5,28]. In addition, there is growing concern that the widespread use of antibiotics such as erythromycin, ciprofloxacin, and tetracycline in veterinary medical practice and as additives to animal feeds (particularly poultry) can select for resistant *Campylobacter* spp., which may be transmitted to humans through the food chain [5,46].

In the Arabian Gulf region, there remains much to be done in understanding the pattern and trends of antibiotic resistance in *Campylobacter* isolates of humans and poultry origin. However, emerging data now shows that *Campylobacter* isolates obtained from both human and poultry sources in Saudi Arabia and Bahrain remain highly sensitive to erythromycin [47, 48]. Evaluation of 250 *Campylobacter* isolates obtained from 2002 to 2006 showed that only three isolates (all of human origin) were resistant to erythromycin, and none of the chicken isolates showed resistance to this antibiotic (Figure 1) [47,48]. Similarly, Sonnevend *et al.* [49] did not detect any resistant isolates when they assessed erythromycin sensitivity in 41 *C. jejuni* isolates obtained from patients in the United Arab Emirates (2002-2004).

These findings imply the continued usefulness of erythromycin as a first-line drug in the Arabian Peninsula. However, responsible use is called for and proper dosing is essential because exposure to sub-inhibitory concentrations of this antibiotic could

potentially enhance the toxigenic effect of

Figure 1: Pattern of antibiotic resistance in *C. jejuni* isolates of human and poultry origin from Bahrain and Saudi Arabia (2002-2006).



A: Erythromycin: Only three isolates (all of human origin) were resistant to erythromycin. None of the isolates of poultry origin was erythromycin resistant.
B. Ciprofloxacin: High levels of resistance which increased from 2002-2006 in both human and chicken isolates.
C. Tetracycline: Increasing trend of resistance in both human and chicken isolates. The resistance was significantly higher in chicken isolates compared to those of human origin ($p \leq 0.01$).

Campylobacter cytolethal distending toxin [50]. In contrast, available data is indicative of high levels of ciprofloxacin resistance in the region. The highest levels were reported in Bahrain (69-85%) and UAE (84%) with 53% resistance documented in a study in Kuwait [47-49,51]. There is evidence of an increasing trend of ciprofloxacin resistance in *Campylobacter* isolates in the region as previous work by Juma and Neringer [52] had reported 50% fluoroquinolone resistance among *Campylobacter* isolates obtained from UAE from 1999 to 2002. Evaluation of isolates obtained between 2002 and 2004 from the same setting showed 85% fluoroquinolone resistance [49]. Data from Bahrain confirms this rapidly increasing ciprofloxacin resistance with 69% of resistant isolates identified in 2002 to 2004 compared with 84% in 2005 to 2006 (Figure 1). Additionally, high levels of ciprofloxacin resistance (80-100%) were also detected in chicken isolates obtained from Bahrain and Saudi Arabia [47]. Quinolone resistance in *Campylobacter* is a rapidly emerging global problem and high levels comparable to those found in Bahrain and UAE have also been documented in Spain (75-88%), Thailand (96%), Hong Kong (85.9%), and India (77.1%) [53-56]. Using the mismatched amplification mutation assay, the Thr-86-to-Ile mutation in the gyrase A gene (*gyrA* gene) was identified as the molecular basis of ciprofloxacin resistance in 80% of resistant isolates studied in Kuwait (23/26) and UAE (35/41) [49]. Based on the partial sequences of gyrA, resistant isolates were found to carry ten distinct alleles, eight of which represented new variants. With PFGE and PCR- RFLP typing, 22.8% of these represented a single molecular type. The widespread use of fluoroquinolones in clinical practice and possible utilization in veterinary practice could be contributing factors for these high levels of ciprofloxacin resistance. These high levels of resistance underscore the need to replace fluoroquinolones as first-line drugs in the treatment of *Campylobacter* enteritis and as empirical therapy for diarrheoa of undiagnosed aetiology in the region.

Available data on tetracycline resistance are from studies carried out in Bahrain [47,48]. These show high levels of tetracycline resistance with an upward increase over time in keeping with other reported data [57]. The level of resistance (as measured by MIC values) was significantly higher in chicken isolates compared to those of human origin ($p \leq 0.01$). This pattern has occurred probably because of decreased use of tetracyclines by clinicians and its persistent use in animal husbandry. However, Avrain *et al.* [58] recently described the natural horizontal transfer of tetracycline resistance gene (*tet*(O) gene) without antimicrobial selection pressure between *C. jejuni* in the digestive tract of chickens, and this is may also explain these high rates of tetracyclineresistance. Indeed, all tetracycline-

resistant *C. jejuni* strains were positive for *tet*(O) gene, while none of the susceptible strains gave a positive PCR result. The occurrence of plasmids in *C. jejuni* isolates varies depending on the geographical area and the origin of the isolate. Of the 100 *C. jejuni* isolates studied by Mazi *et al.* [47], ninety-nine harbored plasmids ranging in size from 15-35kb with the 23kb and 35-kb size being the most commonly identified plasmids. A significant association ($p < 0.01$) between plasmid carriage and tetracycline resistance was observed in chicken isolates but not in those of human origin. These differences in the plasmid profile suggest that the *Campylobacter* strains circulating among humans could have been acquired from sources other than poultry products. The challenge for researchers in the region would be to conduct further work using DNA fingerprinting techniques for the validation of clonal independence of the isolates circulating in chickens and humans.

Laboratory diagnosis

Fecal sample remains the specimen of choice for the isolation of *Campylobacter* spp. in patients presenting with gastrointestinal symptoms. In certain situations, particularly in infants, rectal swabs are also acceptable specimens. However, for this fastidious microorganism, it is imperative to ensure favorable transport and storage conditions including use of transport media in the pre-analytical phase.

Conventional diagnostic methods require that suspected stool specimens are cultured on selective agar at 42^{o}C under microaerophilic conditions for up to 72 hours before a negative report is issued. Only culture plates with colonies showing the characteristic *Campylobacter* morphology and oxidase positivity are then reported as *Campylobacter* spp. However, further identification to the species level requires other tests including growth temperature preferences, antibiotic sensitivity to cephalothin and nalidixic acid, and biochemical tests, mainly the hippurate test. Identification of *Campylobacter* to species level is not performed by most diagnostic laboratories in the region.

The extended and tedious nature of the identification procedures has stimulated research into molecular methods for *Campylobacter* diagnosis. The first report on the application of polymerase chain reaction (PCR) in the diagnosis of *Campylobacter* was described by Oyofo *et al*. in 1992 [59]. Since then several investigators have studied the application of multiplex PCR for the detection and speciation of this pathogen; however, these protocols have been optimized for isolates obtained from pure cultures and artificially spiked stool specimens [60-64]. However, the application of multiplex PCR on bacterial colonies means that conventional culture with all its associated tediousness is still required for the initial identification. We developed a multiplex PCR diagnostic approach for the simultaneous detection and speciation of *Campylobacter* in stool specimen [45]. This method uses a novel combination of genus-specific virulence (*cadF*) genes and species-specific (*hipO* and *asp*) genes with a distinct advantage over previously reported multiplex PCR methods as it can be applied directly on stool specimens from patients and those of poultry origin. Only two previous reports have described the direct application of a multiplex protocol on stool obtained from patients with enteritis and both used primers targeting the *ceuE* gene [63, 65].

This multiplex PCR protocol has the potential to improve the clinical management and epidemiological tracking of *Campylobacter* enteritis. The procedure had a turnaround time of ~6 hours (from DNA extraction to gel electrophoresis) in contrast to the two to five days required for conventional diagnostic methods. Although with an estimated cost of US$3.7 per test it is more expensive than conventional methods (US$0.92 per test), the availability of the laboratory results (showing speciation) to the clinician on the same day could have an impact on clinical management. In addition to its usefulness for clinical diagnostic laboratories, this novel protocol also has potential use for public health laboratories for investigating the epidemiology of *Campylobacter* colonization in poultry flocks and for *Campylobacter* testing in food. Indeed, this protocol has been successfully applied on poultry stool specimens obtained from slaughter-houses in Saudi Arabia and Bahrain. In outbreaks of *Campylobacter* enteritis, this protocol can help to rapidly establish the causative *Campylobacter* species and can also be applied retrospectively on frozen specimens after an outbreak to correctly understand patterns of transmission.

Conclusion

Campylobacter remains a major cause of enteritis globally and within the Arabian Peninsula. A clearer understanding of the molecular characterization of the isolates in circulation and how this impacts the clinical pattern of infection and the immunological response to infection is also emerging. The evidence clearly indicates responsible use of erythromycin,

which appears to remain useful for the treatment of this infection in our region, as well as the need for continuous antibiotic surveillance. New molecular diagnostic approaches offer the promise of enabling rapid identification and speciation of *Campylobacter* that can be of significant clinical impact.

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