

Full Length Research Paper

Occurrence and susceptibility patterns of *Campylobacter* isolated from environmental water sources

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Environmental waters are established sources of *Campylobacter* infections in humans. The aim of this study was to assess the distribution and susceptibility profiles of *Campylobacter* species isolated from irrigation and domestic water sources in Ghana. Samples were pre-enriched with CCDA broth and isolated on mCCDA agar. Isolates were confirmed on API CAMPY kit and with the Kirby-Bauer disk diffusion method to determine the susceptibility patterns of species. Of the 188 water samples analyzed, 42 isolates were confirmed to be contaminated with *Campylobacter* species, giving a prevalence rate of 22.3%. Prevalence of *Campylobacter* in the various water sources were 35.7% in rivers, 26.2% in streams, 21.4% in wells, 9.5% in ponds and 7.1% in boreholes. Sixty four percent (64%) of *Campylobacter* species were *Campylobacter jejuni*, followed by *Campylobacter coli* (19%) and *Campylobacter lari* (14%). Resistance was 100% to the β -lactams, 98% to erythromycin, 48-69% to the quinolones, 45-55% to the aminoglycosides, 71% to trimethoprim sulphamethoxazole, 76% to tetracycline and 90% to chloramphenicol. All the isolates (100%) were multidrug resistant. The presence of multidrug resistant *Campylobacter* species in the different water sources sampled in this study may indicate a significant health risk to humans and animals.

Key words: *Campylobacter*, antibiotic resistance, water sources, Ghana.

INTRODUCTION

Worldwide, *Campylobacter* species are known to be common agents of enteritis in humans, generally regarded as foodborne pathogens. They are frequent residents of the intestinal tract of all food-producing animals and

humans (Duncan et al., 2013). *Campylobacter* are also widespread in the environment, for instance in drinking water, effluents, livestock farms, rivers, streams and wild birds (Jones, 2001; Abulreesh et al., 2005). Water

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sources were previously not recognized to be a major transmission route for campylobacteriosis due to the dormant state of species in water, commonly referred to as viable and non-culturable (VBNC) (Murphy et al., 2006). Nevertheless, *Campylobacter* spp. have been isolated from different sources of environmental water, which include rivers (Ugboma et al., 2012), streams (Carter et al., 1987), ponds (Abulreesh et al., 2005), ground water (Ugboma et al., 2013) and coastal waters (Obiri-Danso and Jones, 1999a). The main source of large *Campylobacter*-associated outbreaks around the world has been linked to untreated or contaminated drinking water (Leclerc et al., 2004). In many tropical developing countries, waterborne associated campylobacteriosis are underreported due to the occasional nature as well as challenges in source attribution of *Campylobacter* infections (Miller and Mandrell, 2005).

In Ghana, a significant urban poor population depends on wells, rivers, ponds and streams as domestic water sources due to inadequate supply of potable water. These water sources may also function as drinking water sources for both domestic and farm animals. Contamination of water with enteric pathogens such as *Campylobacter* poses direct threat to public health as increasing resistance has been reported globally in this organism from different sources of infection. The purpose of this study was therefore to establish whether *Campylobacter* species were present in the various water sources of Ghana, and to evaluate the resistance levels of species to clinically relevant antibiotics.

MATERIALS AND METHODS

The study was carried out in Kumasi, Capital of the Ashanti Region and the second largest city in Ghana. The city is traversed by major rivers and streams which include the Subin, Wiwi, Sisai, Owabi, Aboabo and Nsuben among others. One hundred and eighty eight (188) water samples were obtained during early mornings from major rivers, ponds, wells, streams and boreholes beginning from May 2013 to May 2014. The sampling sites were selected based on the extent of use of the water for domestic and recreational activities. Approximately, 500 mL of surface water was collected into sterile bottles and transported on ice packs to the laboratory in an insulated opaque box.

Sample processing, isolation and identification

About 500 ml of water was filtered through Durapore 0.45 µm filters, diameter 47 mm, with the aid of a suction pump (Edwards RV3). Filters were aseptically transferred using sterile forceps into 30 ml universal bottles enriched with 30 ml of blood-free *Campylobacter* broth (Oxoid CM0963) supplemented with CCDA supplement (Oxoid, SRO 155E) and incubated overnight at 37°C. Enrichment broth was gently shaken and cultured on mCCDA agar (Oxoid CM0689) using a sterile swab stick and incubated in a microaerophilic atmosphere at 42°C for 48 h using Campy Gen (Oxoid CN0025A). Biochemical tests including Gram stain, oxidase and catalase were performed on colonies showing typical morphology of *Campylobacter* spp. Isolates which were small,

curved Gram negative bacilli, catalase and oxidase positive, were further subjected to standard phenotypic tests using API CAMPY (bioMerieux, France) to confirm the species.

Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method was done using Mueller-Hinton agar (Liofilchem-Italy) supplemented with 5% sheep blood; inoculated with 0.5 McFarland suspension and incubated microaerophilically at 48°C for 24 h. Assayed antibiotics obtained from ROSCO (Denmark) included; Ampicillin (10 µg/disc), chloramphenicol (30 µg/disc), ciprofloxacin (5 µg/disc), kanamycin (30 µg/disc), erythromycin (15 µg/disc), gentamicin (10 µg/disc), nalidixic acid (30 µg/disc), tetracycline (30 µg/disc), cephalexin (30 µg/disc), trimethoprim sulfamethoxazole (25 µg/disc), norfloxacin (10 µg/disc), cefotaxime (30 µg/disc) and imipenem (10 µg/disc). The inhibition zones were measured and interpreted according to EUCAST- CLSI 2013 breakpoints for *Campylobacter*. Breakpoints established by EUCAST and CLSI 2013, for enterobacteriaceae were used to interpret the results of norfloxacin, trimethoprim sulfamethoxazole, cefotaxime and kanamycin as CLSI breakpoints for these antibiotics has not yet been established for *Campylobacter*. Quality control was achieved using *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) strains.

Statistical analysis

Percentages were used for the descriptive analysis. Associations were determined using the Chi-square test at a significance level of < 0.05 Stata 14.0 software was used for statistical analysis.

RESULTS AND DISCUSSION

This study recorded an overall *Campylobacter* prevalence of 22.3% from different water sources, with the highest, 35.7% in rivers, 26.2% in streams, 21.4% in wells, 9.5% in ponds and 7.1% in boreholes (Table 1). These levels were lower as compared to the 40.4 and 40.5% reported in environmental water samples from Denmark and England, respectively (Kemp et al., 2005; Ramonaite et al., 2014). Groundwater is however perceived to be free of microorganisms hence the improved quality of water from boreholes, wells and springs (Stanley et al., 1998a). The recorded levels in this study were low as compared to the 52.7% in Nigeria (Ugboma et al., 2013).

The presence of *Campylobacter* in surface waters often relates to recent fecal contamination by avian birds, livestock, farm animal manure runoffs and non-disinfected sewage effluent or septic leakage (Jones, 2002; Abulreesh et al., 2006). Additionally, the absence of piped sewerage systems in most developing countries has resulted in large volumes of untreated or partially treated wastewater ending up in nearby water sources (Danquah, 2010). Higher *Campylobacter* contamination of surface waters have been reported in India (41.5%), in Norway (53.3%), in Nigeria (68.7%) and in Poland (70%) (Popowski et al., 1997; Rosef et al., 2001; Baserisalehi et al., 2005; Ugboma et al., 2012). The observed differences in the contamination levels from the different studies

Table 1. Prevalence of *Campylobacter* species isolated from water sources in Kumasi.

Water source	No. of samples	No. of <i>Campylobacter</i> spp. identified (%)	p-Value
Streams	25	11(26.2)	(Chi-square, df)
Wells	39	9 (21.4)	< 0.0001
Boreholes	17	3 (7.1)	(54.74, 4)
Ponds	87	4 (9.5)	
Rivers	20	15(35.7)	
Total	188	42(22.3)	

Table 2. Distribution of *Campylobacter* species from various water sources in Kumasi.

Water source	No. of isolates	<i>C. jejuni</i>	<i>C. doylei</i>	<i>C. coli</i>	<i>C. lari</i>
Streams	11	7	0	1	3
Wells	9	3	0	6	0
Boreholes	3	2	0	1	0
Ponds	4	4	0	0	0
Rivers	15	11	1	0	3
Total	42	27(64)	1(3)	8(19)	6(14)

C. doylei= *C. jejuni* sub sp. *Doylei*.

could be attributed to isolation by culture methods (Percival et al., 2004) or the use of PCR techniques (Van-Dyke et al., 2010).

The type of *Campylobacter* species found in environmental water sources often relates to the source(s) of contamination (Abulreesh et al., 2006). *Campylobacter jejuni* is the most described from surface waters although *C. coli* and *C. lari* have also been isolated from these sources (Hokajärvi et al., 2013), and its presence is associated with sewage discharges (Obiri-Danso and Jones, 1999a, Jones, 2001).

In this study, over 64% of the isolates from the water sources were *C. jejuni* (Table 2); thus, affirming reports that *C. jejuni* is the most dominant species in surface waters followed by *C. coli* (19%) (Popowski et al., 1997; Rosef et al., 2001; Kemp et al., 2005; Ugboma et al., 2013).

Sources of *Campylobacter* in streams relates to input sources: streams running through pasture lands contain mainly *C. jejuni* with some *C. coli* shed by grazing cattle; agricultural run-offs and effluents from water treatment plants (Popowski et al., 1997; Obiri-Danso and Jones 1999a); or from wild birds, (Jones, 2001). In this study, most of the river and stream water samples were from sources influenced by open defecation (human faeces), untreated sewage, agricultural run-offs; and wild birds (Obiri-Danso and Jones, 1999a). *Campylobacter jejuni* sub. sp. *doylei* has no animal host and it is only humans that serve as reservoirs of this sub species (On, 2005), giving an indication that the *C. jejuni* species and sub species (*doylei*) isolated from the studied rivers may have

come from human origin. Moreover, the 0% recovery of *C. coli* from the sampled ponds and rivers could mean that *C. jejuni* has the ability to thrive in the environment regardless of their fastidious nature, since they have demonstrated aerotolerance, starvation and longer survival in culturable forms than *C. coli* in water (Bronowski et al., 2014). This assertion of *C. coli* susceptibility to environmental stresses was supported by findings where 75% of the *C. coli* were recovered from wells while 12.5% each were obtained from boreholes and streams.

Campylobacter species from the various water sources exhibited high levels of resistance to almost all the antibiotics except imipenem which proved to be effective, 0% resistance (Table 4). Again, 100% resistance was recorded against cephalixin, cefotaxime and Ampicillin which agrees with the work of Baserisalehi et al. (2005) who documented 100% resistance in environmental samples. Resistance against trimethoprim sulfa-methoxazole and tetracycline was 71 and 76%, respectively; however, a higher resistance rate of 96% each has been reported in Malaysia (Adzitey et al., 2012). The 98% erythromycin resistance recorded in this study was far higher than the 1 and 40.8% reported in Malaysia and India. Similarly, the 69% resistance also recorded in this study against ciprofloxacin was higher as compared to the 0% resistance documented by Baserisalehi et al. (2005) and Adzitey et al. (2012). Ciprofloxacin resistance in some European countries especially in the Scandinavian countries was previously reported to be low (0%) or less than 10%; however, rates

Table 3. Multidrug resistance exhibited by *Campylobacter* species from the various water sources.

Water isolates	Total	Multi-drug resistance				
		Stream	Well	Borehole	Pond	River
<i>C. coli</i>	8	1(9.0)	6(66.7)	1(33.3)	-	-
<i>C. lari</i>	6	3(27.3)	-	-	-	3(20.0)
<i>C. jejuni</i>	28	7(63.6)	3(33.3)	2(66.7)	4(100.0)	12(80.0)
Total	42	11	9	3	4	15

Table 4. Resistance and susceptibility profile of *Campylobacter* obtained from water bodies in Kumasi.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
	N=42		
Nalidixic acid	40	NA	60
Tetracycline	19	5	76
Erythromycin	2	NA	98
Ciprofloxacin	14	17	69
Chloramphenicol	10	0	90
Ampicillin	0	0	100
Cefotaxime	0	0	100
Kanamycin	36	19	45
Gentamicin	40	5	55
Norfloxacin	28	24	48
Trimethoprim sulfamethoxazole	29	0	71
Cephalexin	0	NA	100
Imipenem	64	36	0

are presently increasing between 50 and 100% in some European member countries (Mackiw et al., 2012).

Fluoroquinolones and macrolides are recognized drugs of choice for *Campylobacter* infections (Zhao et al., 2010) but this recommendation cannot be admitted in sub-Saharan countries where resistance against these drugs is high.

The high MDR (100%) as shown in Table 3 is a reflection of the poor management of antibiotics in agriculture, human and veterinary medicine which has a rippling effect on environmental water sources. Most hospitals and pharmaceutical industries dispose their improperly treated effluents and refuse into the environment which ultimately ends up in water bodies. Secondly, open defecation, poor sewage systems and the use of some water sources as sewer lines are significant contributors to the high levels of resistance in the environment.

Thirdly, poultry and livestock farmers and crop farmers dispose their expended drug containers and wastes primarily by dropping them into drains and other environmental water sources (Sekyere, 2014).

Conclusion

The widespread distribution of multidrug resistant *Campylobacter* species in the different water sources sampled in Ghana suggest these sources are an important reservoir of antibiotic resistance which has implications in the health of people living in the rural and peri-urban areas of the study region where rivers and streams are their main sources of water supply, including domestic and farm animals exposed to them.

Conflict of interest

The authors declare that there is no conflict of interest.

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