

Antibiotic Resistance in *Campylobacter* Isolated from Patients with Gastroenteritis in a Teaching Hospital in Ghana

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Abstract

Campylobacter is a leading causal agent of bacterial enteritis worldwide, but its prevalence is not well documented in Ghanaian hospitals. This study isolated *Campylobacter* species from patients with enteritis or urinary tract infections attending Komfo Anokye Teaching Hospital and assessed the antibiogram profile of isolated species. Two hundred and two (202) in-patients and outpatients samples of all age groups diagnosed with enteritis or UTI infections were analyzed from May 2013 to August 2013. *Campylobacter* species were detected using selective agar (mCCDA) and confirmed on API Campy system (bioMérieux, France), with disk diffusion method determined the resistance profile of the species. Of the 128 enteritis and 74 UTI patients samples analyzed, 26 and 9 isolates were respectively confirmed as *Campylobacter* spp. giving a prevalence of 17.3% (35/202). Species identified were *C. jejuni* (40%), *C. jejuni* sub sp. *doylei* (2.8%), *C. coli* (37%) and *C. lari* (20%). Resistance was 92.3% - 100% each to erythromycin and the β -lactams, 61.5% - 86.7% to trimethoprim sulfamethoxazole, 92.3% - 93.3% to tetracycline, 46.2% - 80% to chloramphenicol, 0% - 60% to aminoglycosides and 0% to imipenem. Multidrug resistance of 97.1% was detected among species. Empirical treatment of *Campylobacter* enteritis with erythromycin and other common and cheap drugs may result in treatment failure in the face of high level resistance observed among the *Campylobacter* species.

Keywords

Campylobacter, Antibiotic Resistance, Gastroenteritis, KATH, Ghana

1. Introduction

Campylobacter is a major agent of gastroenteritis worldwide, and in developing countries infection has strikingly increased in recent years [1] [2]. Worldwide, *Campylobacter* causes between 400 - 500 million cases of diarrhea each year [3]. *Campylobacter jejuni* (sub sp. *jejuni* and *doylei*) and *C. coli* have mostly been implicated in human infections as *C. lari* and *C. upsaliensis* are less prevalent [4]. Campylobacteriosis is usually mild and self-limiting, but uncommonly associated with infections such as endocarditis, septicaemia, cholecystitis and urinary tract infections [5] [6]. These infections typically warrant treatment as well as infections of the immunosuppressed, pregnant women, children, elderly and those with recurrent symptoms; in such cases the macrolides and fluoroquinolones are the first line drugs prescribed [7] [8]. However, global reports of increasing resistance of *Campylobacter* to the drugs of choice and other clinically important antibiotics from human and animal origin are well established [3] [9] [10]. The resistance situation in developing countries such as Ghana might become worse as a result of widespread and unrestricted use of antibiotics as well as inadequate research into antimicrobial resistance.

In Ghana, diarrhea has been identified as the second most common health problem treated in outpatient clinics [11]. Routine screening of aetiological agents of diarrhea currently does not include *Campylobacter* as evident in the many hospital records in Ghana. In the proper management of diarrhea, a key agent such as *Campylobacter* cannot be ignored. This study reported on the prevalence of *Campylobacter* infections among patients presenting with gastric infections and the resistance profile of isolated species.

2. Materials and Method

Study population and site

This study was undertaken at the Microbiology Laboratory of the Komfo Anokye Teaching Hospital (KATH), Kumasi. The hospital is the second-largest in Ghana and the only tertiary health institution in the Ashanti Region. It is a 1, 200 bed capacity referral hospital for 8 Regions in Ghana. In-patients at the various wards and out patients of KATH who were diagnosed with gastroenteritis or urinary tract infections (UTI) were enrolled in the study from May 2013 to August 2013. Patients of all age groups were recruited.

Sample processing, isolation and identification

Stool and mid-stream urine specimens submitted to the Microbiology Laboratory for bacteriological analysis were processed to obtain our target organism. A loopful of fresh faeces and 0.001 mL of urine were plated directly onto modified charcoal-cefoperazone-deoxycholate agar (mCCDA Oxoid CM0689) supplemented with CCDA selective supplement (Oxoid, SRO155E) using sterile loop and 0.001 calibrated loop respectively. The plates were kept in a 2.5 L anaerobic jar and Campy-Gen gas generating kit (Oxoid CN0025A) introduced to keep the microaerophilic condition at 42°C for 48 hours. *Campylobacter* species were identified by morphological characteristics and biochemically by Gram

stain, catalase and oxidase test. Colonies that were small curved Gram negative, oxidase and catalase positive were further analysed on API Campy to identify to species level (bioMérieux, France).

Antimicrobial Susceptibility test

Antimicrobial susceptibility test was performed by the disk diffusion method on Mueller-Hinton agar (Liofilchem-Italy) supplemented with 5% sheep blood; inoculated with 0.5 McFarland suspension and incubated under microaerophilic condition using Campy-Gen CO₂ generating kit at 42°C for 24 hours [12]. Essayed antibiotics sourced from Rosco (Neo-Sensitabs™, 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 diameter of inhibition zone sizes were measured and interpreted according to EUCAST- and CLSI 2013 breakpoints. Established breakpoints for enterobacteriaceae were used to interpret the results of norfloxacin, trimethoprim sulfamethoxazole, cefotaxime and kanamycin as CLSI *Campylobacter* breakpoints for these antibiotics has not yet been established. Quality control was achieved using *E. coli* (ATCC25922) and *S. aureus* (ATCC25923) strains.

Data Analysis

Descriptive analysis was carried out using percentages. Associations were determined using the Chi-square test at a significance level of <0.05. All statistical tests were two-tailed. Stata 14.0 software was used for statistical analysis.

Ethical Approval

Ethical clearance was obtained from the joint Committee on Human Research Publications and Ethics of the School of Medical Sciences and the Komfo Anokye Teaching Hospital (CHRPE/RC/066/14). Specimens collected and processed were given codes; all identities on samples were removed, which made the patients anonymous.

3. Results

Isolation rate of *Campylobacter* from patients with UTI and enteritis

Of the 128 enteritis and 74 UTI cases, 26 (20.3%) and 9 (12.2%) isolates were respectively confirmed as *Campylobacter* spp. giving a prevalence of 17.3% (Table 1). No significant difference was observed in the isolation rate of *Campylobacter* from enteritis and UTI infections ($p = 0.1403$). All the *Campylobacter*

Table 1. Isolation rate of *Campylobacter* from patients with UTI and enteritis.

Infection	No. samples	No. isolates identified	p-value
Enteritis	128	26 (20.3)	0.140
UTI	74	9 (12.2)	
Total	202	35 (17.3)	

species from enteritis were isolated from patients at the out-patient department (OPD), while 4 of the patients in the ward and 5 from the OPD UTI cases were positive for *Campylobacter*. About 88% (31/35) of cases came from the OPD and 11.4% (4/35) from the Wards.

Species specific prevalence of *Campylobacter* from patients with UTI and enteritis

Campylobacter coli (46.2%) were the dominant species recovered from enteritis followed by *C. jejuni* (38.5%) and *C. lari* (11.5%) but 44.4% *C. jejuni*, 44.4% *C. lari* and 11.1% *C. coli* were recovered from UTI infections. One (1) *C. jejuni sub sp. doylei* was obtained from enteritis but none was found in UTI infections (Table 2).

Demographic distribution of *Campylobacter* infections

Isolation rate of *Campylobacter* was highest in the 10 - 29 (34.3%) age group, followed by 30 - 49 (28.6%) and 0 - 9 (22.8.0%) with age group above 50 recording the least (14.3%). The proportion of female and male patients was 67.6% and 32.4% respectively.

Antibiotic resistance profiles of *Campylobacter* species from patients

Resistance among isolates from enteritis to the beta-lactams (Ampicillin, cefotaxime and cephalexin) was 96% - 100%; as 96% was to erythromycin, 92% to tetracycline, 81% to trimethoprim sulfamethoxazole and 54% to chloramphenicol. Against the quinolones resistance was 23% each to nalidixic acid and norfloxacin and 35% to ciprofloxacin. Resistance to the aminoglycosides was 42% to gentamicin and 8% to kanamycin. Resistance among UTI isolates to the beta-lactams, erythromycin and tetracycline was 100% each, 67% to trimethoprim sulfamethoxazole and 56% to chloramphenicol. Against the quinolones, resistance was 67% each to nalidixic acid, norfloxacin and ciprofloxacin as 44% and 11% was observed respectively against gentamicinand kanamycin. All isolates exhibited 0% resistance to imipenem (Table 3). The difference in resistance levels between enteritis and UTI isolates was highly significant ($p < 0.0001$).

Table 2. *Campylobacter* spp. isolated from patients with UTI and enteritis.

Species	No. isolates	Percentage (%)
Enteritis n = 26		
<i>C. jejuni</i>	10	38.5
<i>C. jejuni sub. sp. doylei</i>	1	3.8
<i>C. coli</i>	12	46.2
<i>C. lari</i>	3	11.5
UTI n = 9		
<i>C. jejuni</i>	4	44.4
<i>C. jejuni sub. sp. doylei</i>	0	0
<i>C. coli</i>	1	11.1
<i>C. lari</i>	4	44.4

Resistance profile of *C. jejuni* and *C. coli* isolates from patients

Resistance among *C. jejuni* strains to the β -lactams and erythromycin was 100% each, to the quinolones, 33.3% - 46.7%, to the aminoglycosides 13.3% - 60%, 93.3% to tetracycline, 80% to chloramphenicol and 86.7% to trimethoprim sulfamethoxazole. Strains of *C. coli* showed resistance of 92.3% - 100% to the β -lactams and erythromycin, 0% - 23% to the quinolones and aminoglycosides, 92.3% to tetracycline, 46.2% to chloramphenicol and 61.5% to trimethoprim sulfamethoxazole. No resistance was observed among *C. coli* strains to nalidixic acid, norfloxacin and kanamycin. All strains of *C. jejuni* and *C. coli* were sensitive to imipenem. Generally resistance was common among *C. jejuni* strains than *C. coli* and the difference was significant, $p < 0.0001$ (Table 4).

Table 3. Antibiotic resistance profile of *Campylobacter* spp. recovered from patients with UTI and enteritis.

Antibiotic	Percentage Resistance			p-value <0.0001
	Species Identified N = 35	Enteritis N = 26	UTI N = 9	
Nalidixic acid	40	23	67	
Norfloxacin	34	23	67	
Ciprofloxacin	46	35	78	
Ampicillin	97	96	100	
Cefotaxime	100	100	100	
Cephalexin	97	96	100	
Kanamycin	9	8	11	
Gentamicin	43	42	44	
Erythromycin	97	96	100	
Tetracycline	94	92	100	
Chloramphenicol	54	54	56	
SXT	77	81	67	

SXT = Trimethoprim sulfamethoxazole.

Table 4. Resistance profile of *C. jejuni* and *C. coli* species.

Antibiotic	<i>C. jejuni</i> N = 15	<i>C. coli</i> N = 13	p-value <0.0001
Nalidixic acid	33.3	0	
Norfloxacin	46.7	0	
Ciprofloxacin	40	23	
Ampicillin	100	92.3	
Cefotaxime	100	100	
Cephalexin	100	92.3	
Kanamycin	13.3	0	
Gentamicin	60	23	
Erythromycin	100	92.3	
Tetracycline	93.3	92.3	
Chloramphenicol	80	46.2	
SXT	86.7	61.5	

SXT = Trimethoprim sulfamethoxazole.

Multidrug resistance in *Campylobacter* isolates from patients

Multidrug resistance (MDR) in this study was defined as resistance to three or more classes of antibiotics. Thirty four (34) out of the 35 *Campylobacter* species were multidrug resistant (97.1%). Isolates from enteritis showed MDR of 96.3% as 100% was observed among UTI isolates and the difference was significant; $p = 0.0434$ (Table 5).

4. Discussion

The 17.3% prevalence obtained in our study is within the documented range of 20% in developing countries [13] and similar to other studies in Algeria (17.7%), Nigeria (16.5%) and Tanzania (18.0%) [14] [15] [16]. However, higher rates have been reported in Bangladesh (26%), Thailand (41%), Nigeria (62.7%) and Ethiopia (72.7%) [1] [2] [17] [18]. Lower rates than in our study have also been reported in Zimbabwe (9.3%) and Egypt (5.8% - 9%) [10] [19]. Abraham *et al.* also reported 6.6% and 12.8% in studies in urban and rural Ghana [20]. Although *Campylobacter* is normally recovered from children less than 2 years in most developing countries [21] [22], this study rather had higher prevalence in the 21 - 30 age group [2]. This may be attributed to the design of this study which did not focus on children with acute diarrhoea and also a reflection of the infection sources which were mostly obtained from the outpatient department which is usually dominated by these age groups in the study hospital. According to Friedman *et al.* two age peaks occur in *Campylobacter* acquisition in developed nations, which are ages less than 1 year and at 15 - 55 years [23]. Secondly, *Campylobacter* infections were more prevalent in female (68%) than in male (32%) patients. In studies by Fitzgerald *et al.* and Friedman *et al.*, *Campylobacter* was more prevalent in male patients compared to females [23] [24]. The results from this study probably speculate the gender distribution of *Campylobacter* infections in patients attending the Komfo Anokye Teaching hospital (KATH). Although no obvious reason has been reported on the impact of gender in *Campylobacter* acquisition, Gillespie *et al.* found that being an infant and a female has an increased risk of acquiring *Campylobacter* infection [25].

Campylobacter jejuni (sub species *jejuni*) and *C. coli* are the most frequently encountered species in human infections [4]. This trend was observed in this study where 80% of our isolates were identified to be *C. jejuni* (43%) and *C. coli* (37%) species. Similar results have also been reported in Uganda, Ethiopia and Egypt [1] [10] [26] but Gwimi *et al.* recovered more *C. coli* (60.6%) than *C. jejuni*

Table 5. Multidrug resistance of *Campylobacter* species from patients.

Infection	Multidrug Resistance			
	P-value	<i>C. jejuni</i> n = 15	<i>C. coli</i> n = 13	<i>C. lari</i> n = 7
Enteritis	26 (96.3)	11 (42.3)	12 (46.2)	3 (11.5)
UTI	9 (100)	4 (44.4)	1 (11.1)	4 (44.4)
Total	35 (97.1)			

Multidrug resistance defined as resistance to 3 or more classes of drugs.

(24.5%) from human samples in Nigeria [2]. *Campylobacter lari* is known to infrequently cause human diseases compared to *C. jejuni* and *C. coli*. In this study, *C. lari* was largely found in UTI infections (44%) as well as patients with enteritis (11.5%). The isolates were from adult patients with age ranging from 62 - 77 years and a 3 year old child. Megraud *et al.* isolated urease positive *C. lari* from adult patients with diarrhoea and a child with appendicitis [27].

Resistance of species to erythromycin was alarmingly high (92.3% - 100%), but macrolide resistance among *Campylobacter* has reportedly been low and stable for a long period of time [7]; nonetheless 31%, 51%, and 79% have been reported from Bulgaria, Singapore, and Nigeria, respectively [28]. Erythromycin resistance in *Campylobacter* has been described as gradual processes that demands prolonged exposure [8], and in agreement with this observation, Newman and colleagues have indicated that drugs such as erythromycin has been on the Ghanaian market for a relatively long period of time [29]. It is therefore reasonable to associate the high level resistance in our study to misuse and abuse of this drug due to the long exposure.

Similarly, high level resistance was observed against the β -lactams (96% - 100%) which is comparable to data from Egypt (100%) [10]. Literature reports suggest that majority of *C. jejuni* and *C. coli* strains are intrinsically resistant to the β -lactam agents caused by the production of β -lactamases which are frequently observed [30]. It can therefore be speculated that perhaps our *C. jejuni* and *C. coli* strains were β -lactamase producing strains accounting for the high resistance.

Resistance among *C. jejuni* and *C. coli* to the quinolones was below 50% which is lower than 72% and 80% documented in Spain, Thailand and Hong Kong respectively; but comparable to rates described in Germany (41% - 46%), USA and Canada (19% - 47%) [3] [31].

Resistance to tetracycline was 92.3% - 100% but 72% has been described in Spain and a much lower rate documented in Ethiopia where tetracycline resistance of 22% has been reported in human isolates [1] [32]. Worldwide, the tetracyclines are a heavily used class of antibiotics both in human and veterinary medicine [33], and in Ghana they are largely applied in animal husbandry leading to its widespread resistance [29] [34], possibly accounting for the high resistance currently observed. All isolates were sensitive to imipenem underlining speculation that carbapenems are an exception to the general β -lactam resistance and considered to be effective also in the treatment of campylobacteriosis [3] [8]; but there is the need for caution in its use; as 17% of the isolates exhibited intermediate susceptibility. Also, the low resistance against imipenem could partly be explained by the high cost resulting in infrequent prescription and less abuse.

Multidrug resistance among our isolates was 97.1% which agrees with reports from China (90%) but higher than rates established in France (37%) and Korea (56%) [35] [36] [37]. High resistance levels discovered in our study may be attributed to misuse and unwarranted prescription of antibiotics by physicians who contribute largely to the growth and spread of antibiotic resistance [38].

Furthermore, self-medication is a common feature of patients attending this hospital due to readily available antibiotics across the counter in pharmaceutical stores, market stalls, by the roadside and from hawkers. This practice generally leads to antibiotic under use (sub-optimal dosages) that invariably increases selective pressure and antimicrobial resistance. Macrolides and fluoroquinolones still remain the drugs of choice for *Campylobacter* infections in some countries, however empirical treatment of patients presenting with *Campylobacter* enteritis at KATH with these drugs may result in treatment failure.

5. Conclusion

The presence of multidrug resistant *Campylobacter* strains present among patients at Komfo Anokye Teaching Hospital indicates that most of the cheap and common antibiotics may not be reliable in the empirical treatment of patients with enteritis caused by *Campylobacter* and other related enteric pathogens and therefore necessary for laboratory confirmation of antibiotics. Nevertheless, imipenem and kanamycin proved to be highly effective. A more extensive, multi-regional study would aid in establishing the extent of *Campylobacter* infections in Ghana.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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