Risk of multi-drug resistant Campylobacter spp. and residual antimicrobials at poultry farms and live bird markets in Bangladesh

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Abstract

[EXSCINDED] Abstract Background Understanding potential risks of multi-drug resistant (MDR) pathogens from the booming poultry sector is a crucial public health concern, particularly for developing countries. Campylobacter spp. are among the most important zoonotic pathogens associated with MDR infections in poultry and human. This study systematically examined potential risks and associated socio-environmental factors of MDR Campylobacter spp. in poultry farms and live bird markets (LBMs) of Bangladesh.Methods Microbial culture and PCR-based methods were applied to examine the occurrence and MDR patterns of Campylobacter spp. at hatcheries, broiler farms and LBMs. Antimicrobial residues in broiler meat and liver samples were detected by advanced chromatogrhaphic techniques. A questionnaire based cross-sectional survey was conducted on socio-environmental factors.Results In poultry farms, Campylobacter spp. was primarily found in broiler cloacal swabs (21/49, 43%), followed by water (8/24, 33%) and broiler meat (8/28, 29%). Remarkably, in live bird markets, Campylobacter spp. was detected in higher prevalence in broiler meat (14/26, 54%), which could have an association with bacterial contamination in water sources (11/21, 52%) and floor (9/21, 43%). Majority isolates of the predominant species, i.e., Campylobacter jejuni (33/47, 70%) and Campylobacter coli (14/24, 58%), were observed to be MDR, showing resistance to amoxycilin, tetracycline and erythromycin, and additionally ciprofloxacin, norfloxacin, streptomycin, and azithromycin. Residual antimicrobials, including oxytetracycline, ciprofloxacin and enrofloxacin, were detected in majority of broiler liver (79%) and meat (62%) samples; and alarmingly, 33% and 19%, respectively, with concentration above acceptable limit. Inadequate personal and environmental hygiene, unscrupulously use of antimicrobials, improper waste disposal, and lack of health surveillance and quarantine facilities of diseased birds were distinguishable anthropogenic risk factors, with local diversity and compound influences on MDR pathogens.Conclusion The observed large-scale occurrence of MDR C. jejuni and C. coli and residual antimicrobials in poultry value chain reflects an alarming situation for public health in Bangladesh. Potential contamination sources of MDR Campylobacter and the combined influences of diverse socio-environmental risk factors, noted in this study, would aid in developing interventions to minimize the increasing risks of poultry-associated MDR pathogens under ‘One Health’ banner that includes poultry, human and environment perspectives.

Background

Campylobacter spp. are among the leading causes of food-borne infections causing human gastroenteritis [1] in both developing and developed countries. Approximately 9.0 and 1.0 million annual cases of campylobacteriosis, mostly due to *Campylobacter jejuni,* have been reported in the European Union (EU) and United States, respectively [2, 3]. Children under 5 years and adolescents aged 15 to 25 years are more susceptible to campylobacteriosis, while immune-compromised people can develop prolonged and severe illness by *Campylobacter* spp. [4]. The common clinical symptoms of campylobacteriosis include fever, abdominal pain, and diarrhea, which are mostly self-limiting, but antimicrobials, particularly, erythromycin, tetracycline, and fluoroquinolones, are often used. *Campylobacter* has been associated with at least 11–21% of diarrhea episodes in children from low

income countries [5]. *Campylobacter* spp., including *C. jejuni,* are also recognized as antecedent cause of particular post-infectious neuropathies, namely, Guillain-Barré syndrome, and autoimmune diseases, including Miller Fisher syndrome, reactive arthritis and irritable bowel syndrome [6]. Gastroenteritis by *Campylobacter* infections showed an increasing trend since the last decade [3]. The cytolethal distending toxin (CDT), consisting of CdtA, CdtB, and CdtC subunits, of *Campylobacter*spp. is one of the major virulence factors which contributes to cell cycle arrest, leading to the apoptosis or necrosis of immune cells, and epithelial cells in the intestine [7].

The gastrointestinal tract of poultry and other birds constitute the primary reservoir of *Campylobacter* spp., and the majority (50 to 80 %) cases of campylobacteriosis in humans is attributable to consumption of poultry products [8]. *C. jejuni* and *C. coli* are the predominant campylobacters in poultry, regardless of whether the animals are sick or healthy. Human populations, especially, toddlers and children, often become infected with *Campylobacter* spp. through contaminated food, and by means of water- and airborne infection from various environmental sources, particularly, as a consequence of poor sanitation and hygiene practice in poultry production and supply chain in developing countries including Bangladesh [5, 9].

Antimicrobial resistance (AMR) has become a serious problem worldwide. Approximately 700,000 deaths have been estimated due to AMR in 2013 throughout the world, whereas, according to a prediction, ca. 10,000,000 people may die in 2050 if no action is taken [10]. Unwise antimicrobial use in poultry farms induces selective pressure on zoonotic pathogens, including campylobacters, to develop resistant strains and has been increasingly recognized as an important public health issue, particularly in developing countries like Bangladesh. Due to lack of hygienic practices and knowledge to manage rampant pollutions in different socio-environmental conditions, majority of the population in Bangladesh is highly prone to health disasters associated with MDR infections from the emergent poultry sector. Since a few studies have observed the occurrence and antibiotic susceptibility patterns of *Campylobacter* spp. in poultry farms, detection of antimicrobial residues, together with socio-environmental risks prevailing in poultry farming and LBMs in Bangladesh have not been adequately explored [11, 12].

Being among the most heavily populated regions, there is always a high demand of broiler meat and eggs production in Bangladesh. Inevitably, the poultry industries in this country are inclined to adopt intensive farming methods, including unjudicial use of antimicrobials. Considering the enormous threats of multi-drug resistant *Campylobacter* spp. and antimicrobial residues from poultry industries in the near future, this study aimed to detect *Campylobacter* spp., their antimicrobial resistance patterns, and the extent of antimicrobial residues in potential sources of poultry farms and LBMs, and explored the associated anthropogenic risk factors.The purpose of this study was to empirically understand the risks of MDR infections by campylobacters, occurrence of antimicrobial residues and inducing socio-environmental factors along the poultry production and supply chain in Bangladesh. This study will assist to publicise systematic in depth screening of the infection sources and transmission pathways and developing participatory approach towards reducing public health hazards from zoonotic pathogens.

Methods

Sampling sites, collection and processing of samples

In three categories (farm, hatchery and LBM) a total of 234 samples were collected during October, 2015 to May, 2016 from 9 broiler farms with a flock size ≥2000, 7 hatcheries and 4 LBMs (located within 10 km^2 of poultry farms and hatcheries) in three sub-districts (Gazipur sadar, Sreepur and Tangail sadar) of two poultry-dense districts (Gazipur and Tangail)) of Bangladesh. During each sampling, three sub-samples (100 g) were randomly and aseptically collected, and combined to provide a composite. Farm samples included broiler meats (BM-F, n = 28), cloacal swabs (CS, n = 49), feed (F, n = 21), and water (W, n = 24). Samples from hatcheries included chick meconium (CM, n = 33). LBM samples included broiler meats (BM-M, n = 27), water (W, n = 21), and floor swabs (FS, n = 21). Moreover, a total of 50 samples, including broiler meat (n = 26, each representing a composite of thigh, breast and drumstick) and liver (n = 24) were sampled from LBMs for screening of antimicrobial residues. After collection, the samples were kept in sterile plastic containers, transported in an insulated foam box with cold chain (temperature, 4–6˚C) and processed within 6 h of collection. A portion (10 g) of each sample was mixed with 10 mL phosphate buffer saline (pH 7.4) and homogenized with a tissue grinder (Seward Medical Ltd, London, UK).

Culture based detection of *Campylobacter* spp.

Aliquots (100 µl each) of the homogenized samples were added onto membrane filter (mixed cellulose ester type, 0.45 µm pore size, 47 mm diameter; Biotech, Germany), which was overlaid on blood agar medium (blood base agar no. 2; Oxoid, UK), supplemented with 5% defibrinated sheep blood, and cultured under microaerophilic conditions at 37ºC for 48 hours, according to Shiramaru et al. *(*2012) [13]. Presumptive *Campylobacter* isolates were subjected to species-specific morphological and biochemical assays, including Gram's staining, motility test, catalase, oxidase, and hippurate hydrolysis, according to standard procedures [6].

PCR-based confirmation of the species identity

DNA template of each isolate of *Campylobacter* spp. was prepared by boiling method described by Hoshino et al. (1998) [14]. In brief, a pure bacterial colony grown on blood based agar was mixed gently in 250 µL distilled water and subjected to boiling, followed by immediate cooling on ice, for 10 minutes each. The tubes were then centrifuged at 10,000X *g* for 10 minutes and the supernatant was collected as DNA template for polymerase chain reaction (PCR). Initially, PCR screening targeting 16S rRNA gene was performed according to Samosornsuk et al. (2007) [15] to confirm whether the strains belonged to the genus Campylobacter. Afterwards, *cdtC* gene based multiplex PCR was done for species-specific (*C. jejuni, C. coli* and *C. fetus)* identification following methods described by Asakura et al. (2008) [16]. DNA templates of *C. jejuni* ATCC33560, *C. coli* ATCC33559 and *C. fetus* ATCC27374 strains were used as

positive controls, and that of *Escherichia coli* ATCC 25922, grown in Luria-Bertani broth (Difco, MI, USA) at 37°C overnight, was used as a negative control. Details of all primers and corresponding PCR amplicon sizes are shown in Suppl. Table 1. PCR products were subjected to gel electrophoresis (1.5% agarose, Invitrogen, USA) and after staining with ethidium bromide (0.5 μg ml^{-1}) and destaining with distilled water, each for 10 minutes, gel images were captured using a UV transilluminator (Biometra, Germany).

Determination of antimicrobial resistance

All *Campylobacter* strains were tested for their resistance pattern by standard disk diffusion method. Eight commonly used antimicrobials with standard doses (μg) were examined: amoxycillin (AMX, 30 μg), azithromycin (AZM, 30), ciprofloxacin (CIP, 5 μg), erythromycin (ER, 30 μg), gentamicin (GM, 10 μg), tetracycline (TET, 30 μg), streptomycin (STR, 10 μg) and norfloxacin (NOR, 10 μg). In brief, freshly grown broth culture (equivalent to 0.5 McFarland turbidity) of each strain was uniformly inoculated, using sterile cotton swab, over the entire surface of Muller Hinton agar (Oxoid, UK), supplemented with 5% defibrinated sheep blood. Afterwards, 3–4 antimicrobial discs were placed in each agar plate and incubated at 37 ℃ for 48 h under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2). The zones of growth inhibition were compared with the zone size interpretative standards as described by the Clinical and Laboratory Standard Institute (2007) [17] and thereby interpreted as susceptible (S), intermediate resistant (I) or resistant (R) to the antimicrobials. *E. coli* strain ATCC 25922 was used as a quality control organism. All data were confirmed by conducting at least two replicates of the disc diffusion experiments.

Detection of antimicrobial residues

A total of 50 samples, including broiler meat (n = 26) and liver (n = 24) were tested for the presence of oxytetracycline, ciprofloxacin and enrofloxacin residues. Solid phase extraction of the samples was performed according to Popelka et al. (2005) [18]. A portion (4 gm) of grinded meat or liver tissues was homogenized in 10 ml phosphate buffer (pH 6.5), and treated with 2 mL trichloroacetic acid (30%, v/v) to fractionate the proteins, followed by centrifugation at 7000X *g,* and sonication in an ultrasonic bath, 15 min each. The supernatant (ca. 2 ml) was treated with formaldehyde (100 μL) at 100ºC for 45 min, transferred to a new tube and mixed with equal amount of diethyl ether for 10 minutes at 25ºC, and the oily top layer was discarded. Extraction with diethyl ether was repeated twice. Afterwards, the extracts were filtered (0.45 μm), dried by evaporation and reconstituted with 2 mL mixture of 99% methanol and acetone (1:1) to obtain the final extract, which was stored at 4ºC.

Standard solutions of residues of three antimicrobial agents, namely, oxytetracycline, ciprofloxacin and enrofloxacin, each >98% purity, were prepared by dissolving 1.0 g powder of each in 5 mL methanol (99%) and kept in the dark at 4ºC until analysis by thin layer chromatography (TLC) within a week following standard procedures [19]. Briefly, TLC silica plates of 0.25 mm thickness (Merck, Germany) were activated at 120°C for 2 hrs before inoculating 50 μl of sample extract or standard solution of antimicrobial

residues. Acetone-methanol (1:1) was used as mobile phase solvent and chromatographs were observed at 256 nm. Each of the samples was analyzed in triplicate. An internal standard and a blank was included after every five samples during analysis by both methods.

Quantification of residual antimicrobials were done by ultra-high performance liquid chromatography (UHPLC) for the meat and liver samples, which showed positive by TLC, following procedures described by Cooper et al. (1998) [20]. Stainless steel column Acclaim 120, C18 (5 μm, 120Å, 4.6 X 250 mm) was used for chromatography (Dionex ultimate 3000 UHPLC). Phosphate buffer solution was prepared by adding di-sodium hydrogen phosphate to 0.2 M potassium di-hydrogen phosphate solution until pH 5.0. HPLC mobile phase constituted of distilled water and acetonitrile (85:15, v/v) for oxytetracycline, and 0.01 M phosphate buffer and acetonitrile (80:20, v/v) for others. Acetonitrile was HPLC grade (Panreac, Italy) and other reagents were of p.a. grade (Merk, Germany). A portion (25 μl) of each sample was injected into chromatographic column, equilibrated with mobile phase, and run at 0.8 ml min^{-1} until the mobile phase ascended 7 cm. Afterwards, the column was air dried and visualized under UV light (λ = 254 nm and 366 nm). Standard controls (2 to 200 μg ml^{-1}) were prepared by serially (2-fold) diluting the stock solution of each antimicrobial. Six replicates of each concentration were assayed to standardize the regression equation (coefficient value >0.99). Identification was done by comparing Rf values of antibiotic standards, i.e., 0.35, 0.80, and 0.97 for oxytetracycline, ciprofloxacine, and enrofloxaine, respectively. Peak area was used for antimicrobial quantification by regression analysis, Y = aX+ b, where Y = component area or height, a = slope and b = intercept of the regression line, and X = estimated amount of antimicrobial. Extraction recovery was evaluated with comparison of peak areas for standard antimicrobial solution to that of the TLC-negative broiler tissue homogenates, spiked with the same standard solution.

Data collection on health safety and hygiene practices

A total of 14 broiler farms, including the 9 sampled farms and additional 5 farms in the same geographical locations were enrolled for survey using SSI questionnaires through participatory methods to understand poultry husbandry associated risks, personal and environmental hygiene, and vulnerabilities to campylobacter infections, antimicrobial usages, and occupational safety. Each SSI was conducted involving at least a couple of representatives from each of the selected farms (n = 14) and responses were recorded in hardcopies. Two teams (A and B), each comprising three experienced veterinarians from the Bangladesh Agricultural University, conducted the SSIs at two phases upon prior individual written consent of the farm managers or farmer owner. Team A collected data from half of the selected farms (n = 7) in the first phase, which were verified by Team B in the second phase, and vice versa for the rest farms (n = 7). A total of 56 SSIs, including two from each farm at each of the two phases (3-month interval), were accomplished.

Data management and Statistical analyses

Data were recorded into Microsoft Excel 10 (Microsoft Corporation, Redmond, WA, USA) spreadsheet from the hard copies and statistically analysed using 'Xact' (ver. 7.21d, SciLab GmbH, St. Yrieix, France) and Statistica (ver. 10.0, StatSoft Inc., USA). Antimicrobial susceptibility profile of the bacterial isolates was compared as histograms. Descriptive comparison of resistant patterns of *Campylobacter* with respect to diversity in sources and/or anthropogenic factors was performed using mean/ median and standard deviation, and also in the form of the box plots. Differences in the patterns and/or occurrence of antibiotic resistance in *Campylobacter* spp. (*C. coli* and *C. jejuni)* were calculated by the Paired Sample T-test. A '*p'* value of <0.05 was considered significant.

Results

Detection and occurrence of *Campylobacter* spp.

Of the 224 samples, a total of 71 (32%) were contaminated with *Campylobacter* spp. as determined by culture-based methods (Table 1). One presumptive *Campylobacter* was isolated from each of the 71 positive samples. When subjected to genus-specific 16S rRNA PCR, all of them produced an expected amplicon size of 1530 bp, confirming their identity as *Campylobacter* spp. None of the samples which were positive for *Campylobacter* spp. were from chick meconium (CM, n = 33) from hatcheries, and feed (F, n = 21) from the broiler farms (Table 1). While detected in approximately one-third (30%, 37 of 122) of the farm samples, with highest occurrence in cloacal swab, followed by water (33%) and broiler meat (29%). *Campylobacter* spp. was found in approximately half (49%, 34 of 69) of the LBM samples. All of the CM samples from hatcheries were found to be negative of *Campylobacter* spp. Among the cloacal swabs (CS, n = 49) from the broiler farms, *Campylobacter* spp. was detected in 21 (43%) samples, with spatial variations, i.e., 52, 43 and 29 % samples at Gazipur sadar and Sreepur, and Tangail sadar subdistricts, respectively. In LBMs of these subdistricts, 44, 33, and 50%, of floor swab environmental samples respectively, were contaminated with *Campylobacter* spp. Analysis of water samples observed higher contamination of *Campylobacter* at the LBMs (overall, 52 %,11/21; with sub-district wise variation of 33–67 %) in comparison to the broiler farms (overall, 33 %, 8/24; with subdistrict-wise variation of 17–42 %). Among the composite broiler meat samples, comprising edible tissues of muscle and liver, *Campylobacter* spp. was detected in 14 out of 27 (52%), and 8 out of 28 (29%) samples from LBM and farms, respectively. This trend of higher contamination of *Campylobacter* in broiler meat samples from LBMs than farms was commonly observed for all three sub-districts (Table 1).

Multiplex PCR assay targeting the *cdtC* gene differentiated the *Campylobacter* isolates into 47 *C. jejuni* and 24 *C. coli,* producing species-specific amplicon of 524 bp and 313 bp, respectively (Suppl. Fig. 1). In both LBMs and farm environment, *Campylobacter* isolates were dominated by *C. jejuni.* Of the 37 isolates from the farm samples, 24 (65%) and 13 (35%) were identified as *C. jejuni* and *C. coli,* respectively. Similarly, out of 34 campylobacters isolated from the market samples, 23 (68%) and 11 (32%) were identified as *C. jejuni* and *C. coli,* respectively.

Antimicrobial resistance patterns in *Campylobacter* strains

The zone of growth inhibition for each strain of *C. jejuni* and *C. coli* was compared with the interpretative standard [17] for each of the selected antimicrobials (Fig. 1, Suppl. Table 2). Of the 47 *C. jejuni* isolates, majority showed resistant to amoxycilin (n = 30, 64%), erythromycin (n = 29, 62%) and tetracycline (n = 24, 51%), followed by ciprofloxacin (n = 17, 36%), norfloxacin (n = 12, 26%), streptomycin (n = 12, 26%), azithromycin (n = 7, 15%) and gentamycin (n = 2, 4%). Similarly, among the 24 *C. coli* strains higher resistance to amoxycilin (n = 13, 56%), erythromycin (n = 11, 46%) and tetracycline (n = 10, 42%), and comparatively lower resistance to other tested antimicrobials, including ciprofloxacin (n = 7, 29%), norfloxacin (n = 8, 33%), streptomycin (n = 6, 25%), azithromycin (n = 4, 17%) and gentamycin (n = 2, 8%), were observed.

A considerable portion of the *C. jejuni* and *C. coli* strains showed intermediate resistance pattern to the tested antimicrobials, ranging from 9 to 28% and 8 to 33 % strains of these two species, respectively (Fig. 1 A-B and Suppl. Table 2). A higher percentage of *Campylobacter* strains isolated from farm than market samples were observed to be fully resistant to most of the tested antimicrobials. However, occurrence of intermediate resistance to the antimicrobials were observed to be significantly higher ($p < 0.01$) in *C. jejuni* and *C. coli* strains, isolated from LBMs, when compared to those of the farm samples (Fig 1 C-F).

Among the isolated strains (n = 47) of *C. jejuni,* 10 (21%) were resistant to two antimicrobial agents, while 11 (23%) were resistant to three antimicrobial agents. Alarmingly, about 20% (n = 9) strains of *C. jejuni* showed resistance against five or more antimicrobial agents, including amoxicillin, erythromycin, tetracycline, norfloxacin and azithromycin/gentamycin (Table 2). In case of *C. coli* strains (n = 24), 4 (16%) and 6 (25%) showed resistance against two and three antimicrobial agents, respectively, while a few, i.e., 1 (4%) and 3 (12%), were resistant to four and five antimicrobial agents (Table 2). Overall, *C. jejuni* strains showed higher percentage (n = 33, 70%) of resistance to at least three antimicrobial agents than that of *C. coli* strains (n = 14, 58%).

Occurrence of antimicrobial residues

Detection of oxytetracycline, ciprofloxacin and enrofloxacin residues by TLC showed their presence in majority of the broiler samples, with higher contamination rate for liver tissues (19 of 24, 79%) than meat samples (16 of 26, 62%). In both kinds of samples, comparatively higher occurrence of oxytetracycline (38 and 31%, respectively) than ciprofloxacin (25 and 19%) and enrofloxacin (17 and 12 %) was observed (Table 3).

UHLP-based quantification of antimicrobial residues in meat and liver samples showed the concentration of oxytetracycline, ciprofloxacin and enrofloxacin ranging from 10 to 155, 25 to 135, and 50 to 115 μg kg^{-1}, respectively (Table 3). Considering the concentration range of these antimicrobials, a significant difference between liver and meat samples was not discernible. However, higher frequency (8 of 24, 33%)

of liver than meat (5 of 26, 19%) samples had antimicrobial residues (Table 3) above the recommended level (100 μg kg^{-1}) for food safety [21].

Environmental health and hygiene practices

Data obtained from SSI based interviews of the poultry handlers from the selected 14 broiler farms are summarized in Fig. 2. Among the anthropogenic factors associated with the occurrence of MDR *Campylobacter,* extensive use of antimicrobial agents was captured in all except one of the farms (Fig. 2A). Majority of the farms (71%) usually discarded the poultry-generated waste into agriculture lands while about one-third (30%) of the farms also discarded poultry faeces into aquaculture ponds as fish feed. Quarantine or isolation of sick birds was practiced in 57% of the farms. Periodic health check up by the veterinary authorities and observation of gastroenteritis were reported for nearly half (45%) of the surveyed farms. Use of disinfectants or cleaning solution to prevent infection from common pathogens and spread of campylobacteriosis, and other gastroenteritis remedies was practiced in approximately one-third (36%) of the farms. On the other hand, regular cleaning of the pots used for feeding and watering, and washing of the floors (of the cages) was done only in small proportion (21 and 14 %, respectively) of the farms. Pre-handling hand washing to minimize cross-contamination between broilers was practiced in only 8 out of 14 (57%) farms. However, hand washing interventions, post-handling and before eating, as part of occupational and personal safety, were practiced by only a minor faction (29 and 21%, respectively) of the farmers. All of the representative farmers and poultry-handlers regularly consumed broiler meat and liver reared in their farms. Gastroenteritis among the poultry handlers were reported in majority of the farms, including 5 of 6 farms reporting campylobacteriosis in broilers, which were associated with compound influences of several anthropogenic factors (Fig. 2B). Comparison of symptomatic and asymptomatic infections in individual farms indicated that diverse combinations of multiple risk factors, concerning hygiene (e.g., hand washing, and proper cleaning), poultry rearing practices, waste disposal and health management, were synergistic socio-environmental drivers of the MDR *Campylobacter* pathogens in poultry sector.

Discussion

Emergence of MDR bacteria from poultry farmsis a major threat to public health, especially in low- and middle-income countries. Poultry industry is among the rapidly growing agro-based enterprises with an annual increasing rate of 20% per annum in Bangladesh [22]. Being the major reservoirs of *Campylobacter* spp., poultry and livestock are mainly responsible for the ongoing spread of campylobacteriosis throughout the world. Campylobacters, particularly, *C. jejuni* and *C. coli* have been recognized as the predominant zoonotic bacteria associated with gastrointestinal disorders in humans since the last decade [3, 23]. Recent investigations have claimed a close association of gastroenteritis caused by these pathogens with the widespread occurrence of malnourished under–5 children in developing countries including Bangladesh [5]. The present study provides a glimpse on the occurrence

and potential risks of *Campylobacter* pathogens and antimicrobial residues at multiple sources of poultry production and supply chain in Bangladesh.

Studies in tropical regions have observed large differences in *Campylobacter* prevalence in poultry samples, e.g., 32 and 65% of broiler flocks in Vietnam and Ecuador [24, 25]. Similarly, the variations in *Campylobacter* occurrence at farms and LBMs in this study may be attributed to the impacts of differential anthropogenic practices and environmental variations. Although not observed, contaminated chick meconium in hatcheries and also supplied feeds in broiler farms may be a potential source of poultry diseases [26]. Higher occurrence of *Campylobacter* in cloacal swabs is in accordance with the bacterial natural colonization of broiler intestines, considered as the primary source of contamination in poultry products and environment [27]. The predomination of *C. jejuni* over *C. coli* among the isolated strains from both the poultry farms and LBMs has been a common trend worldwide [28]. In comparison to broiler farms, the higher occurrence of *Campylobacter* spp. in meat samples of LBMs is probably linked to large-scale contamination of feed water and floor environment, facilitating widespread secondary transmission of this zoonotic pathogen. Even in developed countries, with good farming practices and health interventions, frequentcontamination of poultry products by *Campylobacter* spp. at retail markets and slaughter houses is still a major cause of food-borne illness [30, 31].

Large-scale application of antimicrobials as human and veterinary medicine or prophylactic is imposing an immense challenge to public health. In the context of high population density (>1,200 Km^2) within a small country, the situation is very complex in Bangladesh. High occurrence of MDR strains of *Campylobacter* has been reported worldwide [30, 31]. Likewise, a large fraction of *C. jejuni* and *C. coli* populations in poultry samples in this study has been observed as resistant to commonly used antimicrobials, e.g., amoxycilin, erythromycin and tetracycline. Notably, some of these MDR strains, e.g., ca. 20% of *C. jejuni* strains, have also gained resistance to not only β-lactam but also aminoglycoside, quinolone and macrolide, which is in accordance with previous observations [11]. Depending on geo-socio-climatic variations, diverse resistance patterns of *C. jejuni* and *C. coli,* e.g., in Brazil high resistance for ciprofloxacin, nalidixic acid and tetracycline but low for erythromycin have been observed [32]. The higher frequency of resistant strains in *C. jejuni* than *C. coli* (Table 2) could be related to the overwhelming natural predominance of the former species. However, in comparison to *C. jejuni,* higher proportion of *C. coli* strains have been reported to be resistant to tetracycline and erythromycin in EU and China, respectively [33, 34]. On the other hand, relatively low prevalence of resistant *Campylobacter* strains in market samples than broilers farms (Fig. 1) can be attributable to natural decay of active component of antimicrobials, which are usually applied at the farm level. This inference is also supported by our observation of a significantly ($p < 0.01$) increased abundance of intermediate resistant strains in markets than poultry farms.

The presence of antimicrobial residues, largely unexplored in poultry products, is thought to induce resistance among naturally occurring gut flora and potentially contribute to spreading of MDR strains, eventually health hazards, e.g., gastrointestinal and neurological disorders, hypersensitivity, and tissue damage in animal and human populations [35]. In Ho Chi Minh, Vietnam, ESBL-producing *E. coli* in >50%

of asymptomatic healthy human populations, presumably pre-exposed to residual antimicrobials, coincided with an increasing severity in food-borne diseases [35]. The presence of residual content of antimicrobials in most of the tested poultry meat and liver samples in this study is similar to observations reported from another region in Bangladesh [12]. The higher occurrence of such contamination in liver tissue than meat samples (Table 3) is in accordance with their biological processing within poultry animal. In comparison to oxytetracycline, less frequently observed contamination of ciprofloxacin/ enrofloxacin may be related to their relative use in poultry farms. Considering food safety aspects [21], knowledge on the harmful impacts of antimicrobial residues above the acceptance level in broiler tissues, including liver, need to be translated among both the poultry producers and consumers.

Despite sanitation efforts and control measures, in a limited scale, outbreaks of zoonotic diseases are frequent in Bangladesh [36]. According to this study, the level of hygienic and bio-safety measures, e.g., regular hand washing, use of disinfectants, cleaning of the utensils, and washing of the floors and cages are very poor in poultry farms and markets. Therefore, people working in poultry farms and markets are at high risk of occupational health hazards caused by campylobacter infections. An estimated 20–30% of human campylobacteriosis cases have been attributed to imprudent handling, preparation and consumption of broiler meat [37]. Not only the farmers but also other stakeholders, namely, poultry handlers in the LBMs, storekeepers, restaurant owners and household members are responsible to maintain adequate environmental and food hygiene. This study clearly suggests an imperative need to reduce widespread occurrence of secondary contamination of *Campylobacter* in the LBM environment. Adopting optimal slaughtering process with reduced cross-contamination and proper washing with chlorinated water may effectively reduce bacterial loads on chicken carcasses [38]. Results of this study also point out the need of increased efforts in regular health monitoring and quarantine of sick chicken in poultry farms. People residing at a close proximity of poultry farms may also become vulnerable to MDR pathogens because of the large-scale use as fertilizer of poultry-generated waste, which should be properly treated before discarding to adjacent lands and aquatic ecosystems. However, *Campylobacter* infections in a vast majority of people, particularly in developing countries like Bangladesh often remain asymptomatic [5, 9]. The diversity and compound influences of the potential risks of MDR *Campylobacter* (Fig. 2) need to be explored in more details with systematic and long-term observations of their spatio-temporal variations. Antimicrobial-induced genetic recombination in zoonotic pathogens, including campylobacters, is a major problem to effective vaccination or therapeutic measures in poultry farms. Alternative interventions may include practicing good husbandry with prudent use of antimicrobials, maintaining adequate hygiene and sanitation, and introducing probiotics, prebiotics, antimicrobial peptides, and herbal extracts [39, 40]. Developing management guidelines to combat zoonotic diseases requires systematic risk assessment along with the dynamics and diversity of MDR pathogens, including campylobacters, which are often challenging due to disproportionate vulnerabilities but crucial to participatory management. In this regard, the variety of sources, contamination level, and differential risks of MDR pathogens in poultry farms and LBMs, and inducing socio-environmental factors identified in this study will facilitate to adopting appropriate interventions to tackle health hazards from campylobacteriosis and other zoonotic diseases.

Conclusion

This study, detecting antimicrobial residues in parallel to microbiological and sociological investigation on MDR *Campylobacter* in poultry sources, is the first of its kind in Bangladesh. The observed high contamination of MDR *C. jejuni* and *C. coli* strains in broiler samples and diverse environmental sources at poultry farms, and their magnified occurrence at LBMs, is obviously related to the coinciding poor status of hygiene, bio-safety, and health management measures, reflecting an alarming situation for food safety in Bangladesh. Moreover, the presence of residual antimicrobials in majority of the broiler liver and meat samples, which may also stimulate MDR occurrence, is an emerging hazard to human and animal health. Based on the observed potential risks of MDR *Campylobacter,* it is recommended that not only the farm managers but also more assertively the poultry handlers at markets should be included in behavioural change motivational training programs to adopt preventable measures, including strict maintenance of personal and environmental hygiene, regular monitoring of poultry health and prudent use of antimicrobials. Participatory and holistic surveillance on the transmission dynamics of zoonotic pathogens and adoptable intervention strategies, following 'One Health' approaches, need to be promoted through multidisciplinary collaborations; otherwise, the impacts of poultry-originated MDR bacteria and residual antimicrobials to the food chain would bring appalling health disasters in the near future.

List Of Abbreviations

AMR: Antimicrobial resistance

BM-M: Broiler meat at LBMs

BM-F: Broiler meat at farms

CDT: Cytolethal distending toxin

CS: Cloacal swab

LBM: Live Bird Market

F: Feed

FS: Floor swab

MDR: Multi-drug resistant

W: Water

TLC: Thin-layer chromatography

SSI: Semi-structure interview

UHPLC: Ultra-high performance liquid chromatography

Declarations

Ethics approval and consent to participate

Semi-structure interviews of representative informants were performed upon their prior consent. Letter of approval to conduct the study was received from the Ministry of Fisheries and Livestock, Bangladesh.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analysed during this study are available from the corresponding author on reasonable request. All data generated or analysed during this study are included in this published article [and its supplementary information files]. All the data generated in this study do not in any way allow poultry farms and markets, respondents, or study communities to be identified. Confidentiality of data is maintained anonymously.

Competing interests

The authors declare that they have no competing interests.

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Authors’ Contributions

SBN designed the study, analyzed data and prepared the draft manuscript. MMI acquired the field data and performed microbiological analysis. SMLK, as the principal investigator of the research project, supervised the study, and finalized the manuscript. SY, advised and supervised the analysis. SSI and AHMTA facilitated field- and laboratory-based examinations and contributed in data analysis. All authors read and approved the final manuscript.

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References

1. Nachamkin I, Allos BM, Ho T. *Campylobacter* species and Guillain-Barre syndrome. Clin Microbiol Rev. 1998; 11(3):555-567.https://doi.org /10.1128/CMR.11.3.55.
2. Havelaar AH, Ivarsson S, Lofdahl M, et al. Estimating the true incidence of campylobacteriosis and salmonellosis in the European Union, 2009. Epidemiol Infect. 2013; 141(2): 293-302. https://doi.org/10.1017/S0950268812000568.
3. Scallan E, Hoekstra RM, Angulo FJ et al. Foodborne illness acquired in the United States —major pathogens. Emerg Infect Dis. 2011;17(1): 7. https://doi: 10.3201/eid1701.P11101.
4. Friedman, CR, Neimann J, Wegener HC, et al. Epidemiology of *Campylobacter jejuni* infections on the United States and other industrialized nations. *Campylobacter*. 2000;121-138.
5. Platts-Mills JA, Kosek M. Update on the burden of *Campylobacter* in developing countries. Curr Opin Infect Dis. 2014;27(5): 444. https://doi: 10.1097/QCO.0000000000000091.
6. Nachamkin I. *Campylobacter* and *Arcobacter*. Manual of Clinical Microbiology. American Society for Microbiology. Washington, DC. 2003; 902–914.
7. Yamasaki S, Asakura M, Tsukamoto T, et al. Cytolethal distending toxin (CDT): genetic diversity, structure and role in diarrheal disease. Toxin Rev. 2006; 25, 61–88. https://doi.org/10.1080/15569540500320938.
8. Skarp CPA, Hänninen ML, Rautelin H, et al. Campylobacteriosis: the role of poultry meat. Clin Microbiol Infect. 2016; 22(2):103-109.https://doi.org/10.1016/j.cmi.2015.11.019.
9. Taniuchi M, Sobuz SU, Begum S, et al. Etiology of diarrhea in Bangladeshi infants in the first year of life analyzed using molecular methods. J Infect Dis. 2013; 208(11): 1794-1802. https://doi.org/10.1093/infdis/jit507.
10. O'neill J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Rev Antimicrob Resist. 2014; 20:1-16.
11. Kabir SML, Sumon MH, Amin MM, et al. Isolation, identification and antimicrobial resistance patterns of *Campylobacter* species from broiler meat sold at KR Market of Bangladesh Agricultural University Campus, Mymensingh. J Agric Food Technol. 2014; 4:15-21.
12. Sattar S. Hassan MM. Islam SK, et al. Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh. Vet World. 2014; 7(9): 738-743. doi: 10.14202/vetworld.2014.738-743.

13. Shiramaru S, Masahiro A, Haruna I, et al. Cytolethal distending toxin gene-based multiplex PCR assay for detection of *Campylobacter* in stool specimens and comparison with culture method. J Vet Med Sci. 2012; 74(7):857-862.
14. Hoshino, K., et al., Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic Vibrio cholerae O1 and O139. FEMS Immunol Med Microbiol. 1998. 20(3):201-207.https://doi.org/10.1292/jvms.11-0574.
15. Samosornsuk W, Asakura M, Yoshida E, et al. Evaluation of a cytolethal distending toxin (cdt) Gene-Based Species-Specific Multiplex PCR assay for the identification of *Campylobacter* strains isolated from poultry in Thailand. Microbiol Immunol. 2007; 51(9): 909-917. https://doi.org/10.1111/j.1348-0421.2007.tb03974.x.
16. Asakura M, Samosornsuk W, Hinenoya A, et al. Development of a cytolethal distending toxin (cdt) gene-based species-specific multiplex PCR assay for the detection and identification of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter fetus*. FEMS Immunol Med Microbiol. 2008; 52(2): 260-266.
17. Performance standards for antimicrobial susceptibility testing.17th Informational Supplement document M100-S17. 2007; 32-50. Clinical and Laboratory Standards Institute. Wayne, Pennsylvania.
18. Popelka P, Nagy J, Germuska R, et al. Comparison of various assays used for detection of beta-lactam antibiotics in poultry meat. Food Addit Contam. 2005;22(6):557-562. https://doi.org/10.1080/02652030500133768.
19. Thangadurai S, Shukla SK, Anjaneyulu Y, et al. Separation and detection of certain β-lactam and fluoroquinolone antibiotic drugs by thin layer chromatography. Anal Sci. 2002;18(1):97-100. https://doi.org/10.2116/analsci.18.97.
20. Coope AD, Stubbings GWF, Kelly M, et al. Improved method for the on-line metal chelate affinity chromatography–high-performance liquid chromatographic determination of tetracycline antibiotics in animal products. J Chromatogr A. 1998; 812(1-2):321-326. https://doi.org/10.1016/S0021-9673(97)01290.
21. European Commission. Commission Regulation (EU) No. 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of European Union. 2010; 15, 1–72.
22. Lonkar P, Harne SD, Kalorey DR, et al. Isolation, in vitro antibacterial activity, bacterial sensitivity and plasmid profile of Lactobacilli. Asian-Australas J Anim Sci. 2005; 18(9):1336-1342. DOI: https://doi.org/10.5713/ajas.2005.13.
23. European Food Safety Authority. Scientific Opinion on Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA J. 2011; 9: 2105.https://doi.org/10.2903/j.efsa.2011.2105.

24. Carrique-Mas JJ, Bryant JE, Cuong NV, et al. An epidemiological investigation of *Campylobacter* in pig and poultry farms in the Mekong delta of Vietnam. Epidemiol Infect. 2014; 142(7):1425-1436.
25. Vinueza-Burgos C, Wautier M, Martiny D, et al. Prevalence, antimicrobial resistance and genetic diversity of *Campylobacter coli* and *Campylobacter jejuni* in Ecuadorian broilers at slaughter age. Poult Sci. 2017; 96(7): 2366-2374. https://doi.org/10.1371/journal.pone.0207567.
26. Mohanta MK, Islam MS, Saha AK, et al. Antibiogram profiles of bacterial isolates from poultry feeds and moribund hens in Rajshahi, Bangladesh. Imp J Interdiscip Res. 2016; 2(5): 41-46.
27. Romero-Barrios P, Hempen M, Messens W, et al. Quantitative microbiological risk assessment (QMRA) of food-borne zoonoses at the European level. Food Control. 2013; 29(2): 343-349.https://doi.org/10.1016/j.foodcont.2012.05.043.
28. Kramer JM, Frost JA, Bolton FJ, et al. A *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. J Food Prot. 2000; 63(12): 1654-1659. https://doi.org/10.4315/0362-028X-63.12.1654.
29. Reich F, Valero A, Schill F, et al. Characterisation of *Campylobacter* contamination in broilers and assessment of microbiological criteria for the pathogen in broiler slaughterhouses. Food Control. 2018; 87: 60-69. https://doi.org/10.1016/j.foodcont.2017.12.013.
30. Zhao S, Young SR, Tong E, et al. Antimicrobial resistance of *Campylobacter* isolates from retail meat in the United States between 2002 and 2007. Appl Environ Microbiol. 2010; 76(24): 7949-7956. https://doi: 10.1128/AEM.01297-10.
31. Han X, Zhu D, Lai H, et al. Prevalence, antimicrobial resistance profiling and genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* isolated from broilers at slaughter in China. Food Control. 2016; 69:160-170. https://doi.org/10.1016/j.foodcont.2016.04.051.
32. Ferro ID, Benetti TM, Oliveira TCRM, et al. Evaluation of antimicrobial resistance of *Campylobacter* isolated from broiler carcasses. Br Poult Sci. 2015; 56(1): 66-71. https://doi.org/10.1080/00071668.2014.981796.
33. European Food Safety Authority, European Centre for Disease Prevention and Control. EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. EFSA J. 2015; 13(2):4036. https://doi.org/10.2903/j.efsa.2015.4036.
34. Ma L, Wang Y, Shen J, et al. Tracking *Campylobacter* contamination along a broiler chicken production chain from the farm level to retail in China. Int J Food Microbiol. 2014; 181: 77-84.
35. Babapour AL, Azami L, Fartashmehr J, et al. Overview of antibiotic residues in beef and mutton in Ardebil, North West of Iran. World Appl Sci J. 2012; 19(10): 1417-1422. DOI: 10.5829/idosi.wasj.2012.19.10.180.
36. Samad M. Public health threat caused by zoonotic diseases in Bangladesh. Bangl J Vet Med. 2011; 9(2): 95-120. DOI: https://doi.org/10.3329/bjvm.v9i2.13451.
37. European Food Safety Authority. Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain.

EFSA J. 2011; 9(4): 2105. https://doi.org/10.2903/j.efsa.2011.2105.

38. Pissol AD, Cansian RL. The effect of water pressure and chlorine concentration on microbiological characteristics of spray washed broiler carcasses. Poult Sci J. 2013; 1(2): 63-77.DOI: 10.22069/PSJ.2013.1483.

39. Svetoch E, Stern N. Bacteriocins to control *Campylobacter* in poultry—a review. Poult Sci. 2010; 89(8):1763-1768. https://doi.org/10.3382/ps.2010-00659.

40. Federighi M. How to control *Campylobacter* in poultry farms?: an overview of the main strategies. Poultry Science. 2017;115-132. https://dx.doi.org/10.5772/64846.

Tables

Table 1 Occurrence of Campylobacter spp. along the poultry supply chain (hatchery, broiler farm and LBM) in selected poultry-dense regions in Bangladesh*.

Sub-district, District	Hatchery (CM)	Broiler farm [positive/sample no. (%)]					Live Bird Market [positive/sample no. (%)]				Total
	[positive/ sample no. (%)]	CS	F	W	FBM	Sub-total	BM	W	FS	Sub-total	[positive/ sample no. (%)]
Gazipur sadar, Gazipur	0/15 (0)	11/21 (52)	0/9 (0)	5/12 (42)	3/12 (25)	19/54 (35)	7/12 (58)	6/9 (67)	4/9 (44)	17/30 (57)	36/99 (36)
Sreepur, Gazipur	0/9 (0)	6/14 (43)	0/6 (0)	2/6 (33)	3/8 (38)	11/34 (32)	5/9 (56)	3/6 (50)	2/6 (33)	10/21 (48)	21/64 (33)
Tangail sadar, Tangail	0/9 (0)	4/14 (29)	0/6 (0)	1/6 (17)	2/8 (25)	7/34 (21)	2/6 (33)	2/6 (33)	3/6 (50)	7/18 (39)	14/61 (23)
All	0/33 (0)	21/49 (43)	0/21 (0)	8/24 (33)	8/28 (29)	37/122 (30)	14/27 (52)	11/21 (52)	9/21 (43)	34/69 (49)	71/224 (32)

*CM, CS, F, W, BM, TS, FBM and FS indicate chick meconium, cloacal swab, feed, water, farm broiler meat, transport swab, market broiler meat, and floor swab, respectively.

Table 2 Multi-drug resistance patterns among the poultry-originated strains of C. jejuni and C. coli.

Resistance Category	Resistance patterns*	*C. jejuni* (n = 47)		*C. coli* (n = 24)	
		No. of strains (%)	Sub-total (%)	No. of strains (%)	Sub-total (%)
Against two	AMX-STR	3 (6)	10 (21)	2 (8)	4 (16)
	AMX-TET	5 (11)		2 (8)	
	ER-CIP	2 (4)		0 (0)	
Against three	ER-STR-CIP	5 (11)	11 (23)	3 (13)	6 (25)
	AMX-ER-NOR	3 (6)		2 (8)	
	AMX-TET-CIP	3 (6)		1 (4)	
Against four	AMX-STR-TET-CIP	3 (6)	3 (6)	1 (4)	1 (4)
Against five	AMX-ER-TET-NOR-AZM	3 (6)	5 (11)	2 (8)	3 (12)
	AMX-ER-TET-NOR-GEN	2 (4)		1 (4)	
Against six	AMX-ER-TET-CIP-NOR-AZM	4 (9)	4 (9)	0 (0)	0 (0)
Total		33 (70)		14 (58)	

*Resistant to antimicrobials at standard doses (µg): amoxycillin (AMX, 30 µg), streptomycin (STR, 10 µg), erythromycin (ER, 30 µg), tetracycline (TET, 30 µg), ciprofloxacin (CIP, 5 µg), norfloxacin (NOR, 10 µg), gentamicin (GM, 10 µg), and azithromycin (AZM, 30 µg).

Table 3 Occurrence of antimicrobial residues in meat and liver of broiler chicken.

Antimicrobial agent	Meat samples (n = 26)				Liver samples (n = 24)			
	Positive [n (%)]	Median ± SD (µg Kg^{-1})	Range (µg Kg^{-1})	Above acceptance [n (%)]	Positive [n (%)]	Median ± SD (µg Kg^{-1})	Range (µg Kg^{-1})	Above acceptance [n (%)]
Oxytetracycline	8 (31)	72 ± 52	10 –140	2 (8)	9 (38)	86 ± 38	30 – 155	4 (17)
Ciprofloxacin	5 (19)	93 ± 42	25 –130	2 (8)	6 (25)	90 ± 34	50 – 135	3 (13)
Enrofloxacin	3 (12)	75 ± 29	55 –115	1 (4)	4 (17)	81 ± 23	50 – 105	1 (4)
Total	16 (62)	76 ± 43	10–140	5 (19)	19 (79)	87 ± 33	30 – 155	8 (33)

Figures

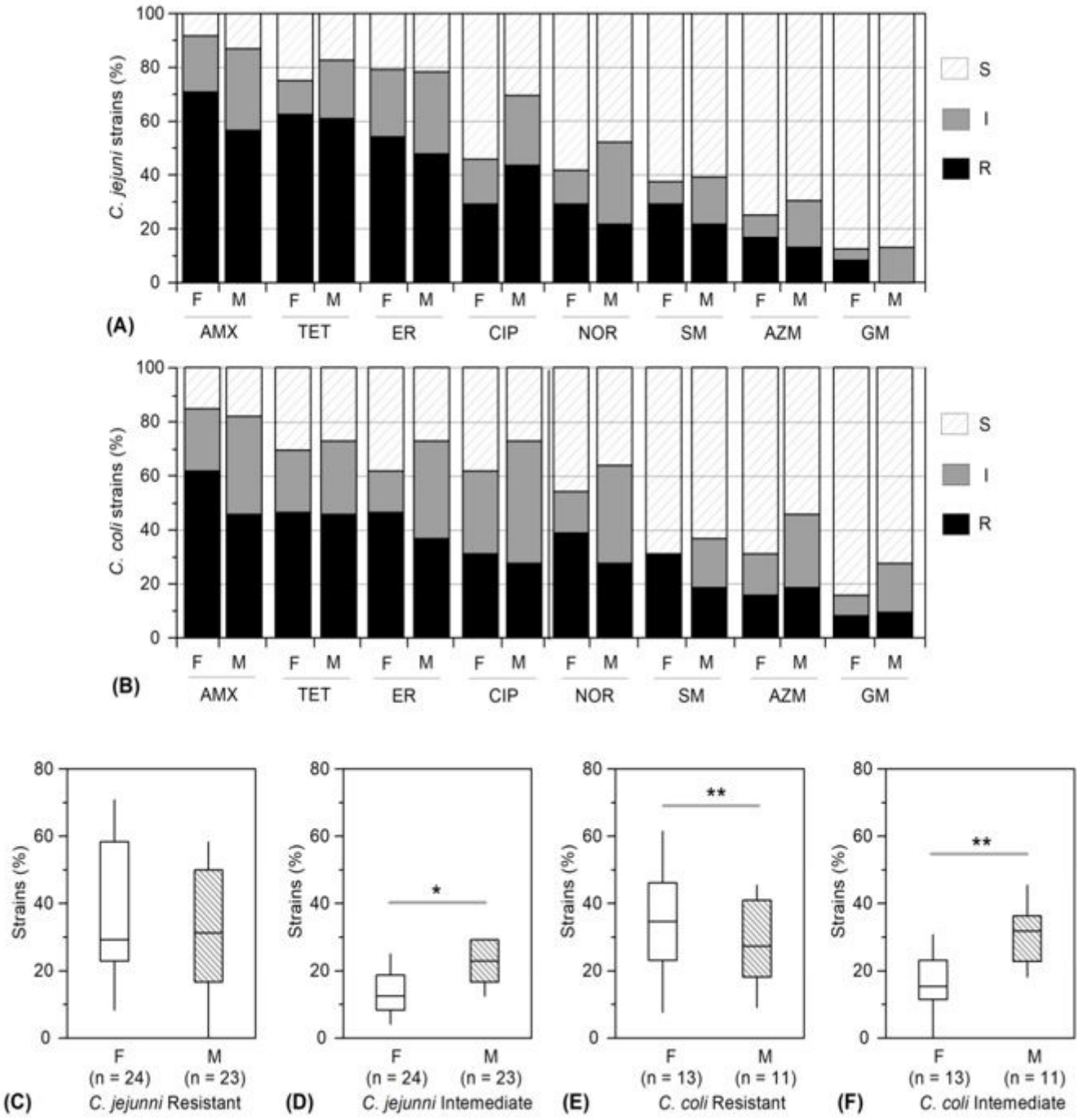

Figure 1

Fig. 1 Comparative occurrence of resistant C. jejuni and C. coli strains isolated from broiler farms (F) and retail markets (M). Eight commonly used antimicrobials at standard doses (µg) were examined: amoxycillin (AMX, 30 µg), tetracycline (TET, 30 µg), erythromycin (ER, 30 µg), ciprofloxacin (CIP, 5 µg), norfloxacin (NOR, 10 µg), streptomycin (STR, 10 µg), azithromycin (AZM, 30 µg), and gentamicin (GM, 10 µg). The bottom and top of the box plots indicate the 25th and 75th percentile. Horizontal lines in the boxes indicate median values and their standard deviations are shown as vertical bars. *p < 0.05 and **p < 0.01, significant differences (paired t test) between mean values of the samples.

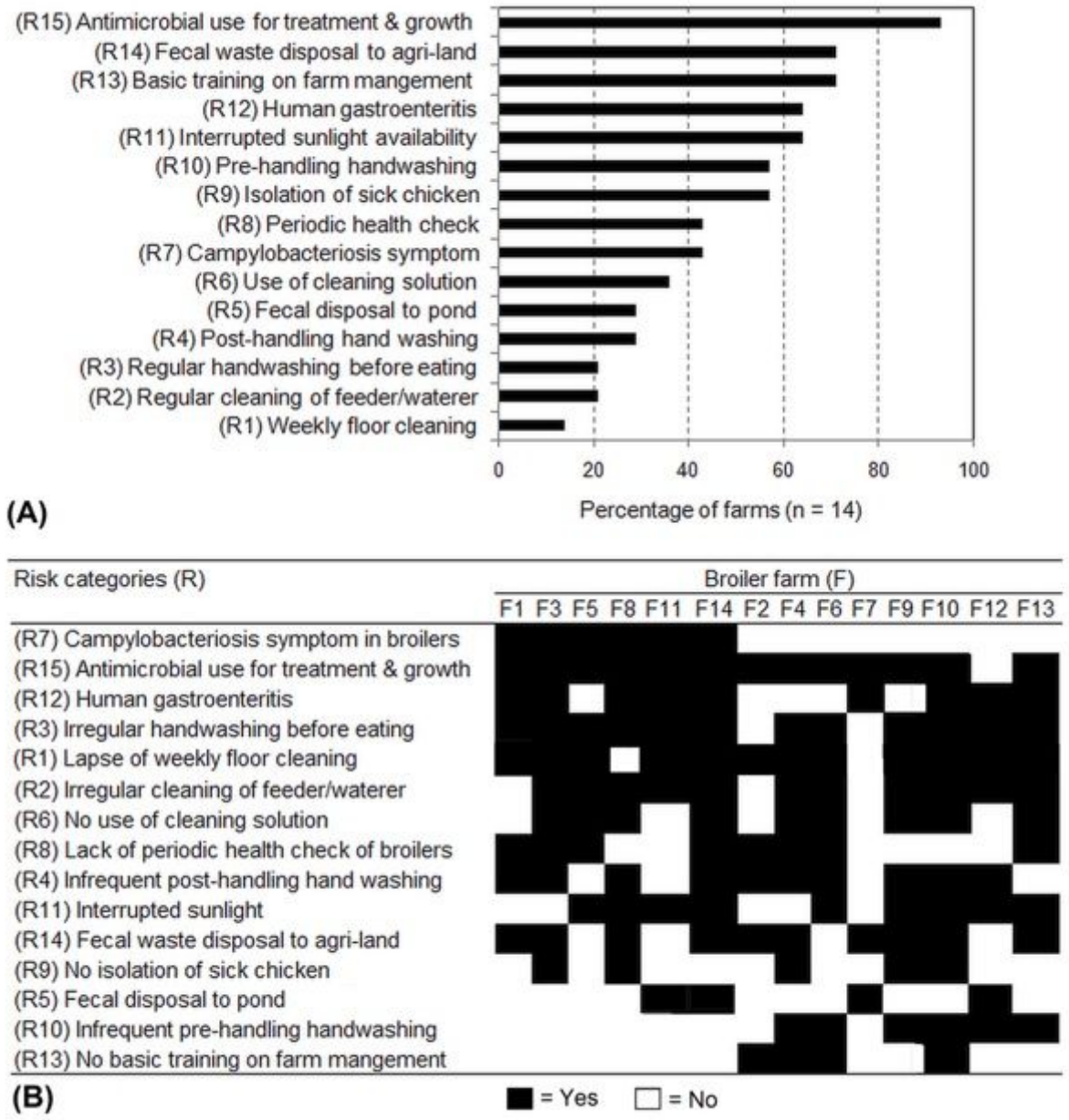

Figure 2

Fig. 2 Anthropogenic factors associated with the occurrence of MDR strains of Campylobacter in the broiler farms. (A) Overall prevalence of the probable risk factors in the selected farms. (B) Individual farm-wise categorization of the risk factors in relation to symptomatic and asymptomatic infections of Campylobacter. Data were obtained at two phases (3-month interval) by interviewing (semi-structured interview) 56 representatives of 14 farms, including 9 (F1-F9) sites examined for contamination of MDR Campylobacter.

Supplementary Files

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