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Understanding antimicrobial resistance in *Campylobacter* isolates from poultry environments in Gujarat, India

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ABSTRACT

Campylobacter, a pathogen responsible for human gastroenteritis on a global scale, is primarily spread through the consumption of contaminated chicken meat. Antimicrobial treatments are commonly required in response to campylobacteriosis, highlighting the risk posed by antimicrobial resistance (AMR) in *Campylobacter* to food safety and public health. Monitoring and understanding AMR trends is crucial for effective risk assessment and development of management strategies. The current investigation examines the physical and genetic traits of AMR in *Campylobacter* species found in the caeca of chickens. Samples were collected from chicken farms and live chicken retail outlets across eight major cities in Gujarat, India. Selective culture from 750 samples found 21 of 250 samples from farms (8.4 %) and 56 of 500 samples from outlets (11.2 %) contained *Campylobacter* spp., confirmed by multiplex PCR and Sanger sequencing. *Campylobacter coli* was most common, detected in 56 samples (7.5%), with *Campylobacter jejuni* detected in 21 samples (2.8%). As per phenotypic assay, all the isolates were resistant to antibiotics ampicillin/sulbactam, followed by azithromycin (94.44%). Genomes were sequenced from a subset of 16 *C. coli* and 2 *C. jejuni* isolates for identification of antimicrobial resistance genes (ARGs). Eleven isolates hosted fluoroquinolone and tetracycline resistance genes. Macrolide resistance genes, such as *macB*, were found in 94.4% of genomes. The results of the current research highlight a high occurrence of ARG carriage in *C. jejuni* and *C. coli*, suggesting that resistance to macrolides, quinolones, and tetracyclines is common. The genotype-phenotype concordance observed was 76.39% whereas, remaining discordance (23.61%) observed was due to the six AMR genes, of which two genes were found truncated length while the remaining genes had complete lengths but had mutations. In-depth examination of the linkage between genetic and phenotypic AMR traits can support development of future strategies and policies to address the growing problem of antibiotic resistance and protect public health.

1. Introduction

Campylobacter coli and *C. jejuni* have emerged as a universal bacterial source of foodborne gastroenteritis in humans globally (Akbar Shahid et al., 2024), affects approximately 550 million people each year, including 220 million children under the age of 5 (World Health Organization (WHO), 2020). The most common cause of bacterial foodborne diseases globally is *Campylobacter*. In high-income nations, the estimated incidence of gastroenteritis caused by *Campylobacter* spp. infections ranges from 4.4 to 9.3 cases per 1000 individuals, according to data from the World Health Organization (Tang et al., 2020). *Campylobacters* are primarily transmitted to humans through insufficiently cooked poultry meat, unpasteurized milk or contaminated water sources, and can cause symptoms such as intense abdominal pain, fever, fatigue, and diarrhea (Lekshmi et al., 2023). *Campylobacter* is a foodborne bacterium that is continuously exposed to a variety of antimicrobial treatments used in the production of food animals (Tang et al., 2021). *Campylobacter* is one of the 12 antibiotic-resistant bacteria that the World Health Organisation (WHO) considers to be the biggest threat to human health. (Tang et al., 2022). A total of 80–90% of

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campylobacteriosis cases globally are caused by *C. jejuni*, which can also cause immunoreactive side effects such as polyarthralgia, Miller Fisher, and Guillain–Barré syndromes (GBS) (Al-Khreshieh et al., 2023; Latov, 2022; Panzenhagen et al., 2021). Due to its higher flexibility, *Campylobacter* can infect a variety of organisms posing a risk to public health, especially the gastrointestinal tracts of people, cattle, poultry, and companion animals (Bunduruş et al., 2023). Considering its survivability at lower temperatures and resistance to the low infectious doses, it is widely categorized as a foodborne pathogen (Wu et al., 2022).

The livestock group, primarily chickens and poultry, acts as a major vector for the spread of *Campylobacter* species (Amjad & Zia, 2023; Gharbi et al., 2023). *Campylobacter* spp. are highly prevalent in the faecal samples from poultry and are also able to withstand disinfection (Gloanec et al., 2022). *Campylobacter* spp. are observed to be resistant to a range of antibiotics, including quinolones, macrolides, tetracyclines, aminoglycosides, and chloramphenicol. The multidrug resistance of such organism has turned out to be a global concern (Lekshmi et al., 2023; Qin et al., 2023). The emergence of resistance to essential medicines like fluoroquinolones and macrolides is especially concerning over the years (Liu et al., 2022). The resistance to fluoroquinolones and macrolides has increased tremendously majorly because of chromosomal mutations leading to acquisition of drug resistance (Conesa et al., 2022; Milton et al., 2020). Extensive study has been carried out on the epidemiology of campylobacteriosis, indicating its prevalence in countries such as New Zealand, Czech Republic and Australia. Although the illness is frequently self-limiting, the rising incidence of AMR presents a significant challenge.

The phenotype-genotype assay for campylobacteriosis has assisted in the diagnosis, ARGs identification, and public health surveillance. Compared to conventional microbiology-based identification methods, a variety of molecular techniques, such as Polymerase Chain Reaction (PCR) and next-generation sequencing (NGS), are used more often (Joseph et al., 2023). Concordance assay for the phenotypic assay performed along with the identification of ARGs hassled to improved management and treatment of infections (Mahdi et al., 2022; Painset et al., 2020). Whole genome sequencing additionally yields the potential to find novel epidemiological markers, improving molecular typing and ability for molecular epidemiology, which is turn useful to discriminate between at random and outbreak-associated *Campylobacter* isolates (Silva et al., 2021; Kong et al., 2023).

The current investigation was carried out to assess the occurrence and characteristics of *Campylobacter* spp. in caecal contents collected from chickens on farms and in retail outlets in Gujarat, India, comparing phenotypic and genotypic assessments for AMR and carriage of ARGs.

2. Materials and Methods

2.1. Sample collection

In June 2021, a cross-sectional study was conducted in eight cities (Ahmedabad, Bharuch, Bhuj, Godhra, Himmatnagar, Rajkot, Surat, and Vadodara) in Gujarat, including broiler chicken farms and live chicken retail shops (Fig. 1). The study included a total of 50 broiler farms and 52 live chicken retail shops. Five broiler chickens were selected randomly from each farm, and five broilers and five desi chickens were chosen randomly from live chicken shops. These birds were euthanised in a humane manner by cervical dislocation. Caeca were surgically removed and sealed using sterilised thread, and then placed in sterilised plastic containers. The samples were promptly moved to a portable fridge set at a temperature of 4 °C and then brought to the laboratory. A total of 250 caecal samples from farms and 500 from real retail shops were collected, resulting in a combined total of 750samples. The number 500 samples for retail shops is because few retail shops had either broilers or desi chickens only.

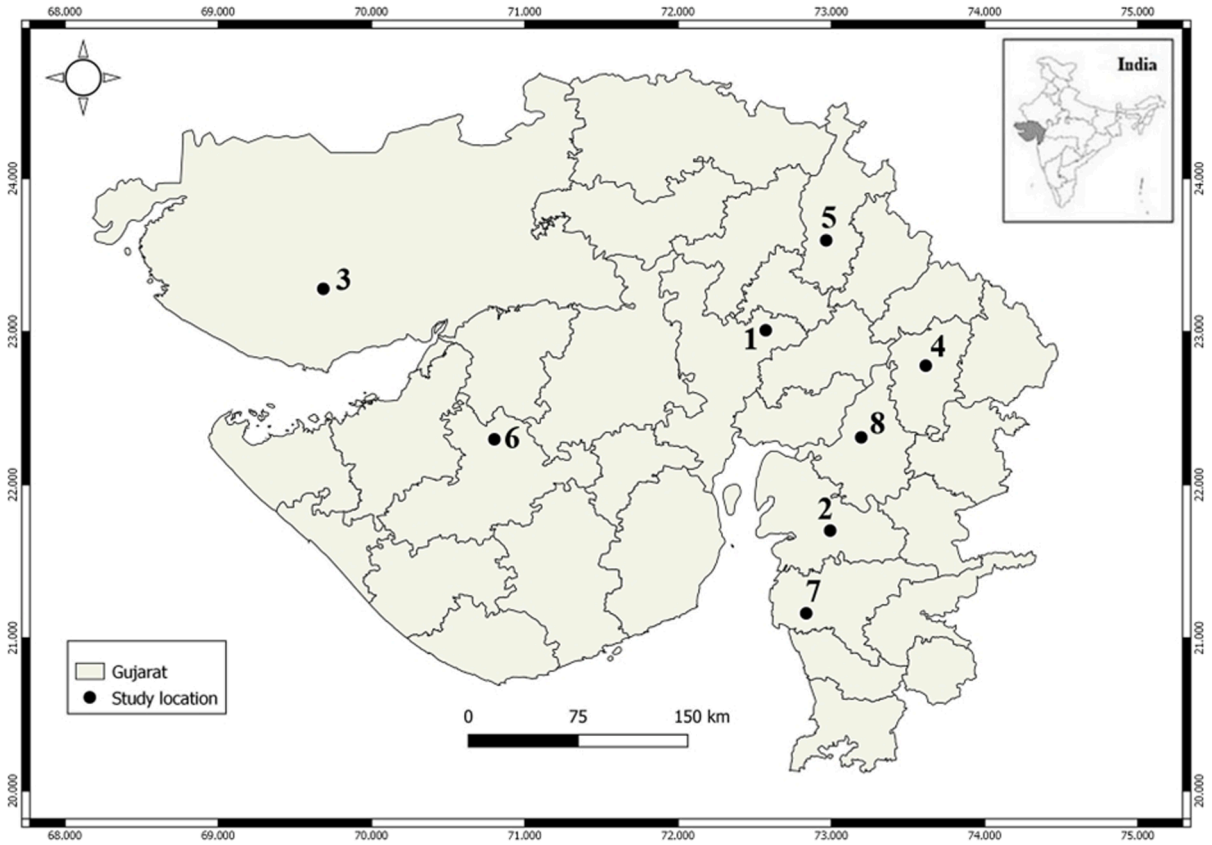

Fig. 1. Map of the study area showing sampling locations in Gujarat State: 1. Ahmedabad, 2. Bharuch, 3. Bhuj, 4. Godhra, 5. Himmatnagar, 6. Rajkot, 7. Surat, and 8. Vadodara (https://www.esri.com).

2.2. Isolation and enrichment of C. coli and C. jejuni

To isolate *C. coli* and *C. jejuni*, caeca were dissected using sterile scalpels to remove the free caecal contents, after which aseptic swabs were used to streak the inner lining of the caeca. Swabs were subsequently streaked onto *Campylobacter* blood-free selective agar plates containing Modified CCDA-Preston agar (Oxoid, UK) under sterile conditions within a biosafety cabinet. The agar medium also contains *Campylobacter* selective supplement IV (Preston selective supplement FD 042) and Tazobactam sodium salt (16 μg/L) procured from Sigma Aldrich. All the plates were incubated under microaerophilic conditions (0.5% CO_2 atmosphere) at 42 °C for 48 hours. One individual, clearly identifiable colony was selected from each plate and subjected to sub culturing and purification.

2.3. Biochemical and molecular characterization

Identification of *Campylobacter* colonies was performed using Gram's staining and confirmed using biochemical assays in accordance with Bergey's Manual of Systematic Bacteriology (Data not provided here). The QIAmp DNA mini kit from QIAGEN was used for DNA extraction. Isolate identity was confirmed by polymerase chain reaction (PCR) using primers that target *Campylobacter* including *C. coli* and *C. jejuni* as described elsewhere (Pang et al., 2002). PCR positive isolates were subjected to additional analysis using MALDI-TOF and 16S rRNA gene sequencing (Gorkiewicz et al., 2003).

2.4. Phenotypic profiling for antimicrobial susceptibility of the isolates

The antibiotic susceptibility (AST) of isolated *C. coli* and *C. jejuni* was determined using the Kirby-Bauer disc diffusion technique. Testing was conducted on Mueller Hinton agar with 5% supplemented sheep blood following instructions provided by the Clinical and Laboratory Standards Institute (Weinstein & Lewis, 2020). ASTs included a panel of 34 antibiotics, representing nine antimicrobial classes (Himedia, India; supplementary Table 1). The multiple antibiotic resistance (MAR) index was computed using the equation specified by (Krumperman, 1983). Specifically, it was calculated by the dividing the total number of antibiotics showing resistance to particular bacterium by total number of antibiotics used. Resistant to antibiotics isolates have been classified as extensively drug resistant (XDR: resistant to at least one antibiotic in all categories except two), pan-drug resistant (PDR: resistant to all tested antimicrobial agents), or multi-drug resistant (MDR: resistant to at least one agent in three or more antimicrobial categories).

2.5. Whole genome sequencing

Sixteen *C. coli* and two *C. jejuni* isolates were chosen for detailed molecular characterization by whole genome sequencing (WGS), including at least one isolate from each city in the region. Genomic DNA was extracted as described above and quantified using a Qubit Fluorometer 4 (Thermo Fisher Scientific, CA, USA) with the Qubit dsDNA broad range (BR) kit. Subsequently, DNA was adjusted to a uniform concentration of 0.2 ng/μL to make desired concentration of total 1 ng DNA as input. Illumina-compatible libraries were generated using the Nextera XT kit (Illumina Ltd., San Diego, CA, USA) and sequencing was performed on a MiSeq platform using 2 × 250 bp paired-end.

2.6. Assembly and annotation of genomes

FastQC (version 0.11.8 for Linux) was employed to evaluate raw sequencing read quality. Trimmomatic was used to remove Illumina adapters and low-quality reads, defined as sequences with an average Phred score of less than 30. Following downstream analyses, the FastQC programme was used to assess trimmed read quality one more time. The genomes were assembled using Unicycler (v0.4.8) (Wick et al., 2017), followed by annotation by Rapid Prokaryotic Genome Annotation (PROKKA) tool (Seemann, 2014). Further, the quality assessment for the assembled genomes was performed using QUAST 5.2.0 tool (Gurevich et al., 2013). The identification of each isolates based on whole genome sequence was carried out based on multi locus sequence typing (MLST) typing using Public Databases for Molecular Typing and Microbial Genome Diversity (PubMLST) (Colles & Maiden, 2012). Also, the completeness and contamination report of the draft assemblies was performed using Microbial Genomes Atlas, version 1.1.2 (MiGA 1.1.2) (http://microbial-genomes.org/). Additionally, specific sequence types (ST) was determined using cgMLSTFinder 1.2 available at https://cge.food.dtu.dk/services/cgMLSTFinder/output.php.

2.7. Antimicrobial resistance genotyping

All the eighteen annotated genomes were analyzed for the presence of antibiotic resistant genes using PATRIC, a bacterial and viral bioinformatics resource center (VanOeffelen et al., 2021) and CARD (Comprehensive Antibiotic Resistance Database) (Alcock et al., 2023). The chromosomal point mutations in the identified ARGs were noted through PointFinder database in ResFinder 4.0 (Bortolaia et al., 2020). However, the ARGs identified were further processed for multiple sequence alignment against reference sequence from NCBI to confirm the mutations.

2.8. Phenotype-Genotype concordance for antimicrobial resistance

The resistance profile from the disc-diffusion assay performed and the ARGs identified from WGS were compared and a concordance/discordance was established. Concordance was satisfied with 100% agreement between the presence/absence of a specific genotype and the corresponding phenotype. Discordance criteria were used when the genotype and phenotype were in disagreement, enhancing approaches for AMR prediction.

3. Results

The current investigation revealed the presence of *Campylobacter* spp. across various cities. A total of 77 isolates were recovered from a pool of 750 samples, indicating a 10.26% prevalence rate of *Campylobacter* spp. For further detailed investigation using whole genome sequencing, 18 isolates were selected and AST profiles of the same were compared. AST analysis revealed a range of resistance patterns across antibiotic classes (Fig. 2). A complete resistance (100%) to ampicillin/sulbactam was observed, followed by azithromycin (94.44%) as per the phenotypic assay i.e. AST. Cephalothin and piperacillin/tazobactam exhibited the resistance rates among beta-lactam antibiotics, at 88.88%, and cefoperazone showed 83.33%. Gatifloxacin and ciprofloxacin demonstrated resistance rates of 88.88% and 83.33%, respectively, to fluoroquinolones. Tetracycline had a resistance rate of 94.44%, whereas azithromycin had the greatest resistance rate of any macrolide at 94%. Teicoplanin and amikacin had the lowest resistance rates, at 50% and 55.55%, respectively.

In addition, it was observed that there was class-specific antibiotic resistance against a range of medicines from different classes, such as beta-lactams, fluoroquinolones, aminoglycosides, non-beta-lactams, macrolides, tetracycline, chloramphenicol, and oxazolidinones. Lincosamides had the highest level of resistance at 88.88%, followed by tetracycline at 83.33%, macrolides at 76.38%, fluoroquinolones at 75.92%, beta-lactams at 73.41%, oxazolidinones at 66.66%, non-beta-lactams at 52.77%, and chloramphenicol 50% (Fig. 3). Among the 18 isolates, 7 (38.88%) displayed MDR, 7 (38.88%) exhibited XDR, and 4 (22.22%) showed pan-drug resistance PDR. The analysis showed that sixteen out of the eighteen isolates had MAR indices more than 0.2, indicating a significant risk of contamination in environments where antibiotics are regularly administered.

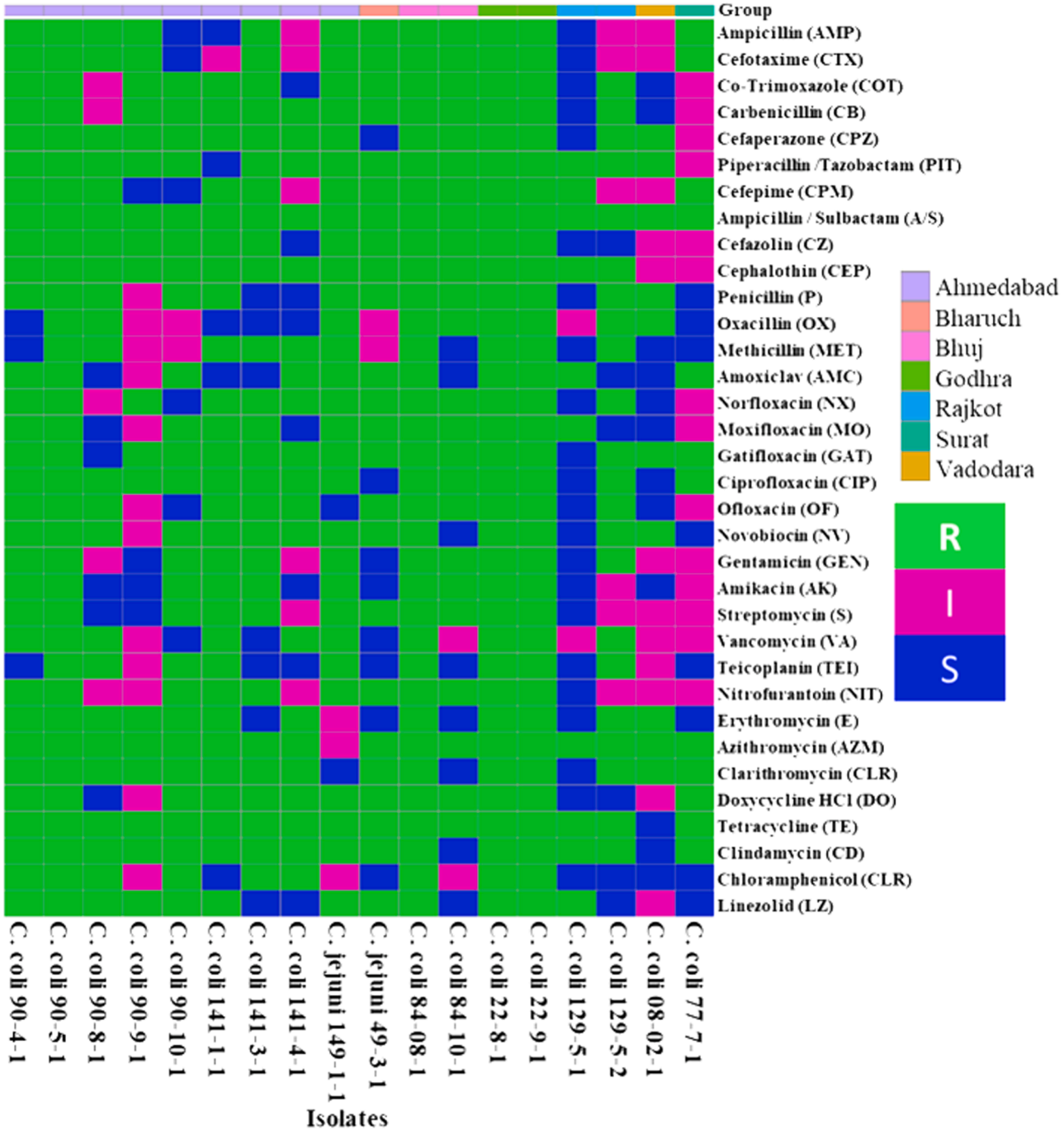

Fig. 2. Phenotypic antibiogram profiles of *Campylobacter* isolates using AST profiling. The R, S and I indicate resistance, susceptible and intermediate antibiotic resistance profile, respectively.

3.1. Genome analysis

Genome of 16 *C. coli* and two *C. jejuni* isolates were sequenced and assembled. Isolate's identity was confirmed using the multilocus sequence typing (MLST) tool available on pubMLST. Among the 18 genomes analyzed, 17 displayed 100% identity with established sequence types and one with 96%. The overall genome quality across all 18 genomes is excellent, contamination is very low, and genome completeness is also very high, as revealed by the results of the Microbial Genomes Atlas (MiGA) (Supplementary Table 2). Both *C. jejuni* has different ST i.e. 11,169 and 23,526. While 16 *C. coli* isolates were classified into eight different STs with ST 357 (n=4) is being highest, followed by 25,693 (n=3), 38,382, 10,602, and 11,262 each has two isolates, and one-one each for ST 7420 and 3590 (Supplementary Table 3).

3.2. Genotypic resistance profiles of Campylobacter spp

Numerous antibiotic resistance genes were detected within all 18 isolates. The beta-lactam resistance gene *oxa-61* was detected in 27.77% of the isolates. *cmeA, cmeB, cmeABC*, and *cmeDEF* were present in all 18 genomes, and *cmeC* was detected in 94.44% of the isolates. Macrolide resistance genes, *macB* and *cmeC* were present in 94.44% of the isolates. A comprehensive assessment of non-beta-lactam resistance revealed the presence of *rpoC* in all isolates. Aminoglycoside resistance gene *gidB* was again found in all of the isolates, while *sat-4, aad(6), ant(6)-I*, and *aph*

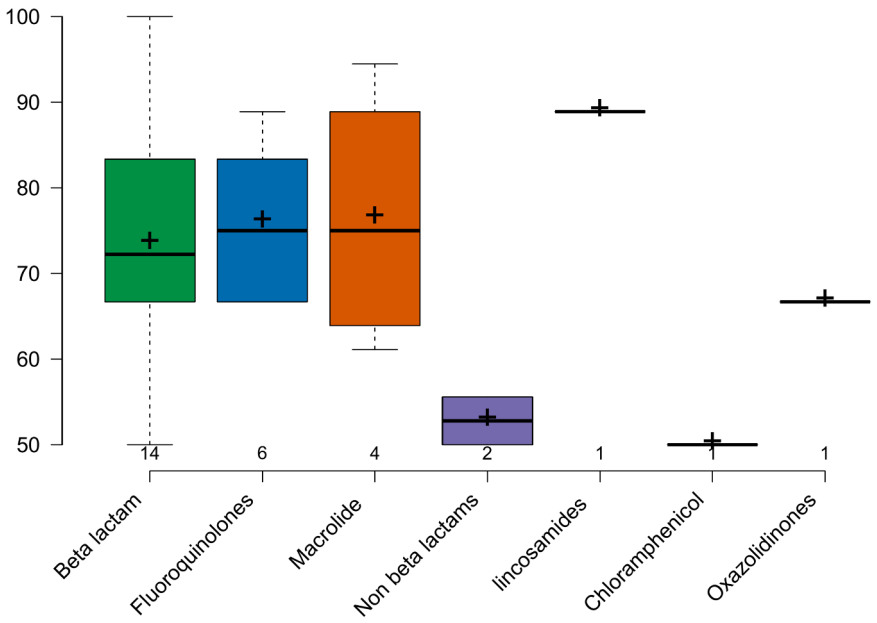

Fig. 3. Resistance profiles of antibiotic drug class in *Campylobacter* spp. based on phenotypic characteristics i.e. AST profiling.

(3′)-III were present in 11% of the samples. Similarly, all isolates exhibited fluoroquinolone resistance genes, *gyrA*, and *gyrB*, being present in 94.44% of the cases. Tetracycline resistance genes *tet(O), tet(W)*, and *ykkCD* were present in 38%, 11%, and 100% of isolates, respectively. Furthermore, Isoniazid-like antibiotic resistance genes *inhA/fabL*, were uniformly present in all the isolates, while *kasA* was detected in 88% of the isolates (Fig. 4).

3.3. Concordance between genotypic and phenotypic resistance profiles, of Campylobacter spp. isolates

The concordance/discordance between phenotypic and genotypic resistance profiles of *Campylobacter spp*. isolates revealed a substantial correlation of AST and genomic profiling (Fig. 5). The overall concordance between phenotype (resistance or susceptible) and genotype (presence or absence of respective genes in the genome) was found to be (76%). Which means that (76%) of the antimicrobial resistance could be explained with genomics studies with cultures showing (9.72%) susceptibility and (73.61%) resistance. The interesting components are remaining (24%) where in the resistance or susceptibility could not be explained with either studies. In (18.1%) isolates despite having antibiotic resistance, genomic mechanisms could not be identified. This opens up newer avenues for detailed investigations about finding novel AMR mechanisms for future. In the small proportion (5.9%) the culture was found to be sensitive despite presence of AMR gene, these phenomena could be explained very well due to lack of promoter or completion of gene.

3.4. Mutation analysis in antibiotic resistant genes

Additional analyses were conducted to investigate instances where the existence of specific genotypes aligns with susceptibility to certain phenotypes, with a focus on examining the occurrence of mutations. Notably, within the macrolide class, a mutation in *MacB* gene in isolate 149–1-1, as well as *CmeA* gene in isolates 8–2-1, 77–7-1, 90–9-1, 141–4-1, 90–8-1, and 129–5-2. Similarly, within the tetracycline group a mutation in *Tet(O)* in isolate 08–2-1., along with mutation in GyrA in isolate 90–9-1 within fluoroquinolone group were also identified. Despite the presence of these genes, phenotypic assay indicated susceptibility toward corresponding antibiotic group. To gain deeper insights, annotated gene sequences were compared from the dataset with reference gene sequences available on NCBI, conducting pairwise sequence alignment. The analysis revealed mutation including, in *macB* gene missense mutation of (P) phenylalanine to (L) leucine at position 445. In the *gyrA* gene, asubstitution of (T) threonine to (I) isoleucine at position 86, and in *tet(O)*missense mutations: (T) threonine to(I) isoleucine at position 227, (V) valine to (A) alanine at position 235, (Y) tyrosine to (C) cysteine at position 295, (I) isoleucine to (L) leucine at position 346, (D) aspartic acid to (E) glutamic acid at position 405, and (Y) tyrosine to (C) cysteine at position 595 were recorded. In the *cmeA* gene, mutations included substitutions of serine (S) to asparagine (N), phenylalanine (F) to leucine (L), and asparagine (N) to serine (S). However, for sample 141–4-1, additional missense mutations were observed in the *cmeA* gene, including asparagine (N) to aspartic acid (D), threonine (T) to valine (V), glutamine (Q) to aspartic acid (D), asparagine (N) to aspartic acid (D), and glutamic acid (Q) to asparagine (N). This approach enabled us to discern significant data about substitutions and mutations that could potentially account for the discrepancy.

4. Discussion

Campylobacters, particularly *C. jejuni* and *C. coli*, are a common foodborne pathogen found in poultry, other animals, and surrounding environments responsible for bacterial gastroenteritis worldwide. The presence of these bacteria in chickens has direct consequences on human health, particularly when individuals consume raw or poorly cooked chickens or other poultry products. Furthermore, antimicrobial resistance (AMR) is frequently seen in *Campylobacter* isolated form the chickens, which is alarming because infection caused by resistant strains are more difficult to cure. Consequently, it is essential for public health and food security to investigate the occurrence of these bad bugs in chickens and monitor AMR genes in them. Therefore, in the present investigation, we isolated Campylobacters from chickens and studied them in detail with specific emphasis of their genetic makeup and observed phenotypes.

The observed prevalence (10.26%) of *Campylobacter* spp. aligns with a related study where a 12.74% prevalence was recorded from 848 samples, with the highest prevalence noted in chicken cecum, followed by chicken meat and slaughterhouses (Pallavi & Kumar, 2014). In Zhejiang Province, China, three chicken farms, two slaughterhouses, and six shops have yielded 100 strains of *Campylobacter jejuni* and reported a novel gene *fexA* for resistant against Florfenicol (Tang et al.,

Fig. 4. Genotypic resistance profiles of *Campylobacter* spp. where the green colour represents presence, and pink colour represents absence of that AMR gene in the genome of that isolate.

2020).

Another parallel study focused on poultry meat and carcasses reported a similar prevalence of 12.79% (Andrzejewska et al., 2015). Yet, this observed prevalence is very less as compared to other studies. Recently studies have reported 46.2% (retail chickens in Colombia) (Ortiz et al., 2024), 29.6% in animal products with poultry being the highest one (47.8%) among others in a worldwide meta-analysis study (Zbrun et al., 2020), 36.17% in chicken meat in South Korea (Je et al., 2023), and 40.5% in cloacal swab samples in Bangladesh (Hasan et al., 2020)prevalence of *Campylobacter*. However, it is important to note that prevalence or occurrence also depends on several factors, including chicken breed, farm management practices, sample type surveyed, season, environmental conditions and others (Andritsos et al., 2023; Rama et al., 2024).

Furthermore, 18 *Campylobacter* isolates were subjected to a phenotypic investigation in order to assess their susceptibility to a wide range of antibiotics belonging to different antimicrobial classes. MAR profiles, revealing a conspicuous pattern of multi-drug resistance among the tested isolates. Alarmingly, sixteen isolates in the current investigation surpassed this threshold (MAR >0.2), underscoring the pervasive nature of antibiotic resistance among the *Campylobacter* isolates (Tawakol et al., 2023). Pathogen's ability to resist more than one antimicrobial agent has become a notable concern and thus has led to the classification of antibiotic resistance pattern into three distinct categories: MDR, XDR, and PDR. A significant fraction of the isolates, precisely 38.88%, manifested MDR and XDR, and a smaller yet significant proportion of

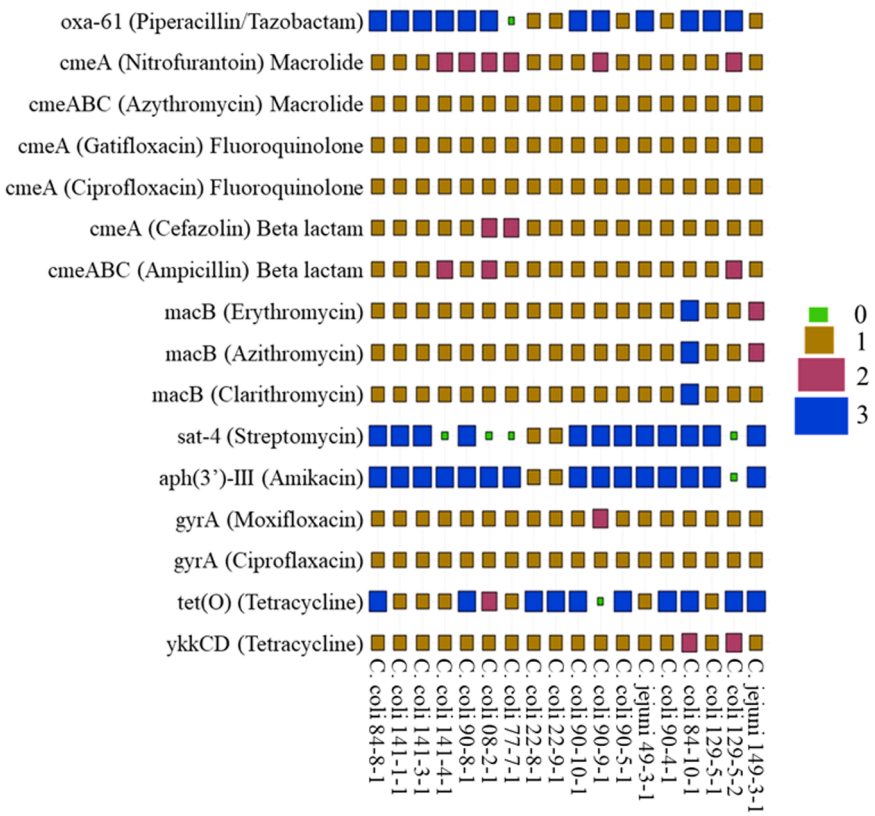

Fig. 5. Concordance of phenotypic and Genotypic resistance profiles of *Campylobacter* spp. isolates. (Concordance criteria 0 = Phenotypically susceptible and genotypic ally gene absent and 1 = Phenotypically susceptible and genotypically gene present) (No-Concordance criteria 2 = Phenotypically susceptible and genotypically gene present and 3 = Phenotypically resistance and genotypically gene absent).

22.22% showcased PDR. In contrast to the present study, another studies, have reported higher MAR index and MDR. (Tawakol et al., 2023) have reported a staggering 72% of isolates exhibited MDR across three to five antimicrobial classes, with a MAR index oscillating between 0.55 and 0.77 Similarly, (Shakir et al., 2021) with 61.1% isolates exhibit MDR. In another comparative studies aa range of MDR isolates have been reported for MDR, 97.7% (Santos-ferreira et al., 2022), 90.7% (Kanaan & Mohammed, 2020), and 64.5% (Tang et al., 2020). A recent study from India also highlights high MAR, 0.11–0.78 with 54.4% isolates are MDR (Suman Kumar et al., 2023). These statistics highlight the widespread and growing concern of antimicrobial resistance in chicken products and thus food security worldwide.

Furthermore, to understand the resistance mechanism, whole Genome Sequencing (WGS) was carried out to thoroughly analyze the AMR profile of 18 *Campylobacter* spp. isolates. The current analysis determines the beta-lactam resistance gene (*OXA-61*) in 27.77% of isolates, which is similar to the findings of a previous study in which the *blaOXA-61* gene was observed in 21.4% (9/42) of beta-lactam-resistant organisms (Béjaoui et al., 2022). Another study found the AMR genes of *blaOXA-61 is 70.27%* (Tang et al., 2022). Another study in China have reported, concurrence of *optrA* and *fexA* in *Campylobacter* isolates from pig (Tang et al., 2021).

The current investigation also focuses on to the *CmeABC* efflux pump, a critical player in conferring resistance to *β*-lactam, macrolides, and fluoroquinolones drugs (Gibreel et al., 2007). Every isolate in the study carried the genes *CmeA, CmeB*, and *CmeABC*, while the *CmeC* gene was identified in 94% of the instances. In a similar study, *CmeABC* in multidrug resistance is corroborated by literature, spotlighting its role in thwarting the efficacy of an array of antibiotics (Gibreel et al., 2007). This study in C. *coli* isolates the presence of genes *sat-4, gidB, aad(6), Ant (6)-I*, and *Aph(3)-III,* conferring resistance to aminoglycosides is 11%. In similar study the AMR genes *aph(3′)-III* (39.64 %), *ant(6)-Ia* (29.73 %), *aadE-Cc* (5.41 %), *aph(2″)-If* (27.03 %), *aac(6′)-aph(2″)* (8.11 %), were detected in 111 isolates (Tang et al., 2022).

The exploration of tetracycline resistance genes unveiled presence of *Tet(O)*, in 38%, *Tet(W)* in 11% and *YkkCD* across all the isolates. Compared to this study, a similar investigation has reported high prevalence of tetracycline resistance in *C. jejuni* and *C. coli* isolated from poultry farms and further *TetO* gene was found in 93.7% of these isolates (Awad et al., 2023). In the case of fluoroquinolone resistance genes, all isolates, including those carrying *CmeA, CmeB, CmeABC*, G*yrA*, and *GyrB*, showed a 100% presence of these genes. In a parallel study (Wanja et al., 2023), the prevalent occurrence of resistance determinant *GyrA* was consistently found in 61% of all isolates, with a little fluctuation between 62.1% and 60.8% in *C. coli* and *C. jejuni*, respectively.

This study further investigated a relationship between phenotypic and genotypic resistance patterns of *Campylobacter* spp., unearthing a pronounced positive correlation between genetic compositions and observed antibiotic resistance behaviors. A focused examination revealed a 33.33% genotypic concurrence for *Oxa-61* and a compelling 88.88% for *CmeA* with Cefazolin in the realm of beta-lactam resistance genes. A similar trend was noted with *CmeABC*, aligning at 83.33% with ampicillin. In this study, we observed strong correlation for macrolide and fluoroquinolone classes. Specifically, the genes *cmeA* were associated with resistance to both nitrofurantoin (66.67%) and gatifloxacin (100%), while the genes *cmeABC* were strongly linked to resistance to azithromycin (100%) and ciprofloxacin (100%). Similarly, results have been reported by the study carried out by Tang et al., (2022). where they have reported a high level of AMR with ciprofloxacin (93.69 %) and nalidixic acid (93.69 %) being the highest. Furthermore, in *macB*, gene with resistance rate of 94.44% for erythromycin and 100% for clarithromycin. In *gyrA* gene moxifloxacin resistance rate was (94%). In

current research work, concerning aminoglycoside resistance genesillustrated a full concurrence, with *sat-4* and *APH (3′)-III* marking a 33.33% and 16.6% alignment with streptomycin and amikacin, respectively. In the context of tetracycline resistance, *Tet(O)* and *YkkCD* demonstrated a level of variety in coexistence, with a prevalence of 38.88% and 88.88%, respectively. In a similar study, tetracycline showed 98.8% concordance between phenotypic and genotypic characteristics i.e. presence of the *Tet(O)* (Whitehouse et al., 2018).

In this study, we also established a criterion to assess the agreement between genotypic and phenotypic traits. A comprehensive investigation was undertaken, specifically examining instances when the existence of certain gene/s and mutation/s corresponds to a vulnerability to AST profile data. The focus within the macrolide class unveiled mutations in the *macB* gene. The specific mutation was identified in isolate 149–1-1 from Ahmedabad, where a mutation leads to 445-phenylalanine to 445-leucine substitution. These findings suggest a clear link between mutation in the *macB* gene macrolide resistance. The primary mechanisms of resistance in *Campylobacter* to macrolides and fluoroquinolones, two essential drugs in clinical setting are chromosomal target mutations and active efflux. This has been corroborated by a previous study within the fluoroquinolone class of antibiotics (Gibreel, 2006). A mutation involving a threonine-to-isoleucine substitution was detected in the *gyrA* gene. A high-level resistance to ciprofloxacin has been documented in a similar study (Ge et al., 2005). This resistance was specifically associated with a mutation at the Thr86Ile within the same *gyrA* gene. But it's important to notice that the alterations found at positions 121 and 196 has been reported for resistance and susceptibility, respectively (Lehtopolku et al., 2011).

5. Conclusion

This study emphasizes an urgent necessity of addressing the threat of antimicrobial resistance (AMR) in *Campylobacter* spp. within the poultry system, caused by the prevalent and frequent negligent utilization of antibiotics. The study shows occurrence of *Campylobacter* spp., specifically *C. coli* and *C. jejuni*, in chicken cecum samples from various cities in Gujarat. A comprehensive analysis of the entire genome revealed a wide range of genes and sometimes mutations in the gene/s associated with resistance, indicating a complex and diversified pattern of antimicrobial resistance (AMR). Thus, whole genome sequencing (WGS) improves our capacity to trace *Campylobacter* and is a great tool for monitoring antimicrobial resistance (AMR) strategy under the One Health concept. These findings are crucial for public health, since they not only define the scope and complexity of antimicrobial resistance (AMR) in *Campylobacter* but also emphasize the necessity for strong, strategic treatments. To prevent the emergence of resistant strains, preserve public health, and assure food safety, it is critical to implement enhanced surveillance, exercise careful use of antimicrobials, and implement comprehensive regulatory reforms.

Ethical considerations

This study was approved by Institutional Animal Ethics Committee of Anand Agricultural University (approval number: AAU/VET/IAEC-32/ABT/329/2020, dated: 31/07/2020).

Data availability statement

The whole genome sequences of the *Campylobacter* isolates have been deposited in Bio project: PRJNA1185018.

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Ethical Statement - Studies in humans and animals

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CRediT authorship contribution statement

Sadik Dantroliya: Writing – original draft, Investigation, Formal analysis. **Monica Chavan:** Software. **Ramesh Pandit:** Writing – review & editing, Validation, Data curation. **Chinmayi Joshi:** Supervision. **Fiona Tomley:** Project administration, Investigation, Funding acquisition, Conceptualization. **Damer Blake:** Writing – review & editing, Data curation. **Richard Stabler:** Methodology. **Chaitanya Joshi:** Project administration, Investigation, Funding acquisition, Conceptualization. **Madhvi Joshi:** Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.100740.

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Further reading

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