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Antibiotic Resistance Profiles of *Campylobacter* species Isolated from Raw Milk Sold by Street Vendors in Chennai

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ABSTRACT: *Campylobacter jejuni* is a leading cause of bacterial gastroenteritis in humans, with rising concerns over its resistance to commonly used antimicrobial agents. This study aimed to evaluate the antimicrobial resistance patterns of *Campylobacter* spp. isolated from raw milk samples collected from street vendors in Chennai. A total of 47 isolates were tested against ampicillin, azithromycin, chloramphenicol, ciprofloxacin, doxycycline, gentamicin, kanamycin, norfloxacin, penicillin G, and tetracycline using in vitro susceptibility testing. The results indicated that all *C. jejuni* isolates were 100% sensitive to chloramphenicol. Additionally, 90% of isolates remained sensitive to gentamicin and kanamycin. However, resistance was observed against azithromycin (6.7%), ciprofloxacin (15.6%), norfloxacin (13.3%), doxycycline (40%), tetracycline (55.6%), and ampicillin (51.1%). Notably, all Campylobacter isolates exhibited complete resistance (100%) to penicillin G. These findings highlight the prevalence of antimicrobial resistance in *C. jejuni*, emphasizing the urgent need for continuous monitoring and the implementation of rational antimicrobial use strategies to control the spread of resistance in food borne pathogens.

Keywords: *Campylobacter jejuni* – antimicrobial resistance – raw milk – street vendor.

INTRODUCTION

Thermotolerant Campylobacter spp. represent a significant global burden of foodborne disease (Epps *et al*., 2013; EFSA, 2019). There has been a surge in campylobacteriosis infections across a wide range of countries (WHO 2013; EFSA, 2019). Campylobacter species, particularly *C. jejuni* and *C.coli*, stand as a leading cause of acute gastroenteritis in humans (Kaakoush *et al*., 2015). Through Meta-analysis, it has been shown that the handling and consumption of chicken meat presents a large risk for human Campylobacter infection (Domingues *et al*., 2012). According to EFSA (2010), chicken handling, preparation, and consumption are estimated to contribute to 20–30% of campylobacteriosis case. In addition, it is also recognized that Campylobacter can be transmitted through raw milk, raw red meat and contaminated produce (Mohammadpour *et al*., 2018). An acute gastrointestinal infection, Campylobacteriosis is characterized by severe abdominal pain, fever, nausea, headache, muscle pain, and diarrhoea. The symptoms persist for 5–7 days with an incubation period of 3–5 days. As reported by Mead *et al*. (1999); Kate *et al*. (2020), Campylobacter is responsible for 17.3% of all food borne bacterial infections. *Campylobacter jejuni* is the major cause of human Campylobacteriosis (85-95%) and *C. coli* accounting for the remaining 5-15% cases (Friedman *et al*., 2000).

Nowadays antibiotic resistance in food borne pathogens is a significant global concern. The use of antibiotics to prevent diseases like mastitis in cows can contribute to the development of microbial resistance to the antibiotics commonly used in treatments of human beings. Research has indicated geographical variations in the frequency of resistance of these organisms to certain drugs, particularly tetracycline (Karmali *et al*., 1981; Buck *et al*., 1982).

Antimicrobials are generally employed in food animals both as therapeutic agents and growth promoters. Their use has been vital in ensuring the high levels of productivity achieved in recent decades, leading to a reduction in both mortality and morbidity and thereby promoting animal well-being. However, the overuse and misuse of antimicrobials can result in the development of antibiotic-resistant bacteria in food animals, which may then be transmitted to human, presenting a significant public health risk (McEwen and Fedorka–Cray 2002).

MATERIALS AND METHOD

Samples. A total of 3175 raw milk samples were collected from local street vendors in Chennai city of Tamil Nadu, the fourth largest metropolitan city in India for analysis in this study. Out of 47 presumptive isolates, 45 were biochemically identified as *Campylobacter jejuni* and the remaining two as *Campylobacter coli*. The isolates were then analyzed for their antibiotic resistance patterns.

Invitro antibiotic sensitivity test. A 200 µl volume of pure cultures of *Campylobacter i*solates, cultivated in Brain Heart Infusion (BHI) broth, was spread-plated onto BHI agar plates under aseptic conditions. Antibiotic resistance was determined using the disc diffusion method with commercially available antibiotic discs (Hi Media), which included the following antibiotics viz. Ampicillin (A) – 10 µg, Azithromycin (At) – 15 µg, Chloramphenicol (C) – 30 µg, Ciprofloxacin (Cf) – 5 µg, Doxycycline (Do) – 30 µg, Gentamicin (G) – 10 µg, Kanamycin (K) – 30 µg, Norfloxacin (Nx) – 10 µg, Penicillin (P) – 10 units, and Tetracycline (T) – 30 µg. The plates were incubated at 42°C for 48 hours. The resistance criteria were based on guidelines from the National Committee for Clinical Laboratory Standards (NCCLS), USA. If no zone of inhibition appeared around the antibiotic discs, then the Isolates were considered as resistant.

RESULTS AND DISCUSSIONS

The antibiotic resistance patterns of the isolates against the antibiotics used in this study was presented in Table 1 and Fig. 1.

In vitro sensitivity testing revealed the following findings that all the *Campylobacter jejuni* isolates were 100% sensitive to chloramphenicol. Forty one isolates of *C. jejuni* were sensitive to kanamycin and gentamicin, except for 4 isolates (8.96%). Additionally, 51.1% of the isolates were resistant to ampicillin 6.7% to azithromycin, 15.6% to ciprofloxacin, 40% to doxycycline and 55.6% to tetracycline. All Campylobacter isolates were 100% resistant to penicillin G.

The two isolates identified as other *Campylobacter* species were highly susceptible to chloramphenicol, ciprofloxacin, gentamicin, and norfloxacin. However, they exhibited 100% resistance to penicillin G and 50% resistance to ampicillin, ciprofloxacin, doxycycline, kanamycin and tetracycline. In conclusion, the most effective antibiotics against *Campylobacter* spp. were chloramphenicol, azithromycin followed by gentamicin and kanamycin.

All the strains were completely susceptible to chloramphenicol, which is consistent with the findings of Avrain *et al*. (2003). The most common resistance observed was to doxycycline (40%), which was in agreement with the report by Sato *et al*. (2004). *C. jejuni* showed a 55.6% resistance to tetracycline, which aligns with the findings of Sato *et al.* (2004), who also reported high tetracycline resistance rates (45%) in *Campylobacter* species. Further, these results were supported by the studies of Nachamkin (1994); Gaudreau and Gilbert, (1998); Padungtod *et al*. (2006).

Similarly, three isolates (6.7%) exhibited resistance to azithromycin, which aligns with the findings of Englen *et al*. (2007). Additionally, 8.9% of the isolates showed resistance to kanamycin, which is in correlation with the moderate resistance observed in three Campylobacter species by Miranda and Lage (2007).

The findings of this study revealed a ciprofloxacin resistance rate of 15.6%, which contradicts the earlier report of Luber *et al*. (2003), who observed a resistance rate of 45.8% in *C. jejuni* isolates from livestock and 45.1% in human isolates in Germany. However, Englen *et al*. (2007) reported a much lower resistance rate (2.3%) to ciprofloxacin in *C. jejuni* strains. Nonetheless, the findings of this study are comparable to the 17.2% resistance to ciprofloxacin reported by the CDC (2006).

The current study found that 51.1% of *Campylobacter* isolates exhibited resistance to ampicillin. In contrast, Rozynek *et al*. (2005) reported a lower resistance range of 13–21% in both human and chicken strains in Poland. Additionally, all *Campylobacter* isolates demonstrated complete (100%) resistance to penicillin G, aligning with the findings of Miranda and Lage (2007); Ema *et al*. (2021).

A study characterizing 541 *Campylobacter* isolates collected from various livestock species across different stages of the North Carolina production chain revealed a high prevalence of antimicrobial resistance. Specifically, 90.4% of the isolates were found to carry genes associated with resistance to at least one antimicrobial agent, while a substantial portion (43%) demonstrated resistance to multiple antibiotics (Hull *et al*., 2021).

Hailu *et al.* (2021) studied the prevalence and antibiotic resistance of *E. coli* O157, *Salmonella*, *L. monocytogenes*, and *Campylobacter* in northeastern Ohio small-scale farms using dairy cattle or poultry manure as fertilizer between 2016 and 2020. The overall pathogen prevalence was 19.3%, with *Campylobacter* at 8%, *L. monocytogenes* at 7.9%, *E. coli* O157 at 1.8% and *Salmonella* at 1.5%. Significantly more pathogens were found in farms using dairy cattle manure (87.7%) compared to those using poultry manure (12.2%). A substantial portion (57.3%) of *Campylobacter* isolates displayed multidrug resistance, notably to aminoglycosides, tetracycline, and penicillin.

The increased resistance to commonly used antibiotics may be attributed to their extensive and indiscriminate use in treating udder infections. McEwen and Fedorka-

Cray (2002) suggested that the majority of resistant microorganisms emerge due to genetic changes, either through mutations or the transfer of genetic material. This is followed by a selection process, primarily driven by the uncontrolled use of antimicrobials.

Variations in antimicrobial resistance observed in Campylobacter across different food animal sources might be influenced by differences in susceptibility testing methods, variations in drug usage practices, or other unmeasured factors related to management or exposure. Nevertheless, monitoring antimicrobial resistance in bacterial pathogens from food animals is crucial for developing effective antimicrobial use strategies in food animal production.

Table 1: Antimicrobial Resistance of *Campylobacter* Isolates by Species.

Sr. No.	Antibiotics	*C. jejuni*	*C.coli*	Total
1.	Ampicillin (A) – 10 mcg	23 (51.1%)	1 (50%)	24 (51.1%)
2.	Azithromycin (At)-15 mcg	3 (6.7%)	0	3 (6.4%)
3.	Chloramphenicol (C)–30mcg	0 (0%)	0	0
4.	Ciprofloxacin (Cf) – 5 mcg	7 (15.6%)	1 (50%)	8 (17.0%)
5.	Doxycycline (Do) -30 mcg	18 (40.0%)	1 (50%)	19 (40.4%)
6.	Gentamicin (G) – 10 mcg	4 (8.9%)	0	4 (8.5%)
7.	Kanamycin (K)--30 mcg	4 (8.9%)	1 (50%)	5 (10.6%)
8.	Norfloxacin (Nx)-10 mcg	6 (13.3%)	0	6 (12.8%)
9.	Tetracycline (T)-30 mcg	25 (55.6%)	1 (50%)	26 (55.3%)
10.	Penicillin (P) – 10 units	45 (100%)	2 (100%)	47 (100%)

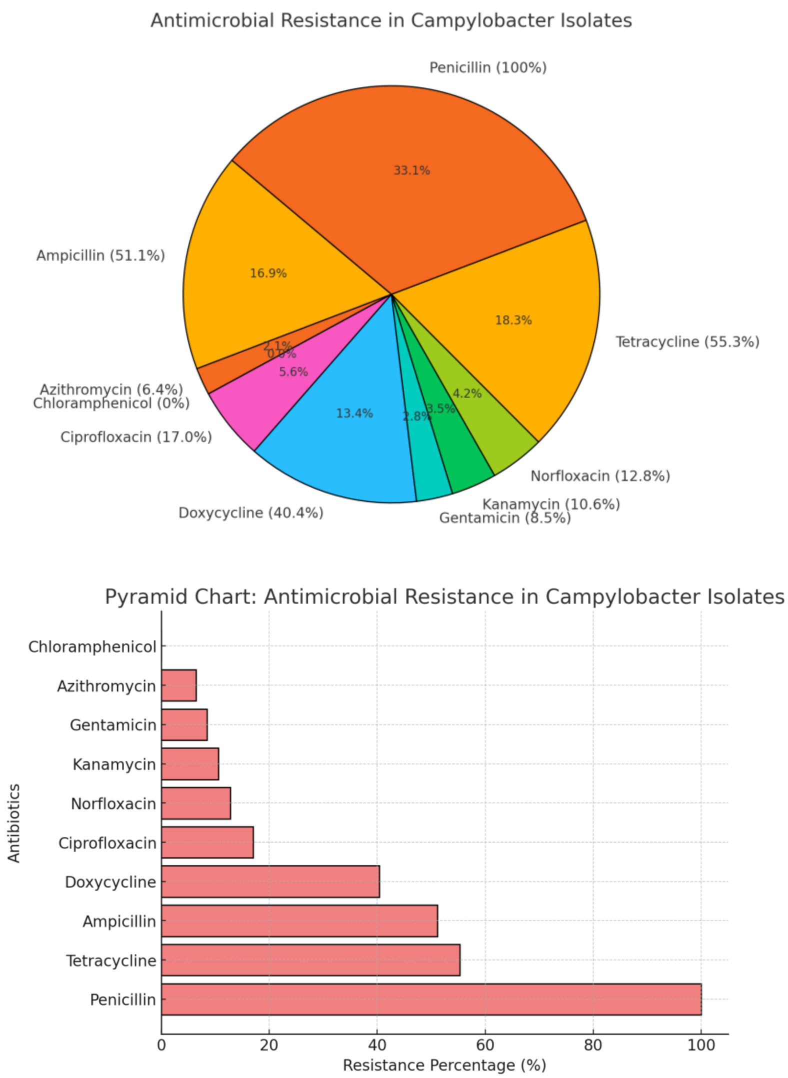

Fig. 1. Antibiotic resistant pattern of *C.jejuni* isolates.

CONCLUSIONS

The findings of this study suggest that antimicrobial resistance in *Campylobacter* spp. is present in milch animals in Chennai, with distinct resistance patterns observed between *C. jejuni* and *C. coli*. Additionally, variations in antimicrobial resistance were noted among *Campylobacter* isolates from milk samples collected from cows in different zones.

FUTURE SCOPE

Future studies could expand upon this research by investigating the genetic mechanisms underlying the observed antimicrobial resistance in *Campylobacter jejuni* isolates. Whole-genome sequencing could identify specific resistance genes and mutations, providing a deeper understanding of how these bacteria are developing and spreading resistance. Furthermore, a larger sample size encompassing a wider geographical area and different types of milk samples (e.g., pasteurized, packaged) would provide a more comprehensive picture of resistance prevalence. Investigating the potential link between antimicrobial use in dairy animals and the emergence of resistant *Campylobacter* strains would also be valuable.

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