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Research Article

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *CLOSTRIDIUM PERFRINGENS* FROM VARIOUS MEAT SAMPLES IN ANAND, GUJARAT

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Abstract: Gram-positive, spore-forming *Clostridium perfringens* is a common bacterium. It can produce enterotoxins in the small intestines of people and domestic animals, contaminating a variety of retail meat products and resulting in food poisoning. We examined into to the prevalence and antimicrobial resistance of *C. perfringens* in carabeef, chicken, mutton, and chevon that shoppers in Anand, Gujarat, purchased from retail meat market shops. 200 meat samples resulted in a total of 31 *C. perfringens* isolates, with poultry having the highest incidence (24%) preceding chevon (16%), mutton (10%), and carabeef (10%). Using the agar disc method, the antimicrobial resistance of the isolates was assessed. Resistance to cephoxitin (61%) was found, followed by moxifloxacin (52%), tetracycline (48%), vancomycin (42%), gentamicin (36%), and ofloxacin (32%). It's remarkable to note that 10 of the 31 isolates exhibited multidrug resistance, or resistance to more than three distinct antibiotic classes.

Keywords: *Clostridium perfringens, Prevalence, Retail meat, Antimicrobial resistance, Multi-drug resistance*

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Introduction

One of the most pervasive spore-forming, quickly proliferating pathogenic bacteria is *Clostridium perfringens* [1]. Numerous human and veterinary disorders are caused by *C. perfringens*, which is known to pose a serious threat to public health [2]. As a result, food regulators in several nations have established the tolerance limit for the presence of *C. perfringens* in raw meat products. For example, India has a zero-tolerance policy for meat products that are consumed raw, and the USA has a performance standard of no more than one log growth during a stabilisation step (cooling) after heat treatment [3,4]. Based on the production of six major toxins, *C. perfringens* is categorised into seven toxigenic categories, A to G: alpha (α), beta (β), epsilon (ε), iota (ι), enterotoxin (CPE), and NetB. To describe the isolates' potential risk and track contamination more accurately in various food manufacturing phases, it is essential to identify the toxin type of *C. perfringens* isolates [5]. Infection by living bacterial cells and their toxins both play a significant role in the development of gastroenteritis in the host when *C. perfringens* causes a toxic, widespread food-borne illness [6]. Many antibiotics, including ampicillin, tetracycline, chloramphenicol, metronidazole, and imipenem, have been used prophylactically in the livestock industry in several nations to reduce the economic losses imposed on by these diseases [7]. Despite growing public awareness of the need to combat antimicrobial resistance to improve public health, research into the antimicrobial resistance of *C. perfringens* from a variety of sources, including the most frequent source of infection in meat samples has been largely inactive over the past ten years [8]. The antimicrobial resistance of *C. perfringens* isolates to the most used antimicrobial drugs was assessed using the agar disc method in this study, which also looked at the prevalence of *C. perfringens* in retail meat samples in Anand, Gujarat.

Material and methods

Collection of Samples

From September 2020 to December 2021, 200 retail meat samples, including 50 each of carabeef, chicken, mutton, and chevon, were bought from wholesale marketplaces (collections of wholesale establishments selling meat samples directly from producers) in Anand, Gujarat, India. Each sample of meat was either raw or processed (chopped). In sterile screw-capped glass tubes containing transport medium, meat samples from various animals and birds were placed after being collected in sterile polyethylene bags and chilled using ice packs. Each bag had the code number and other information about the sample written on it. All samples were delivered to the lab in ice pack containers and tested for *C. perfringens* within 24 hours.

Detection and isolation of *C. perfringens* in meat samples

With some adjustments, the ISO 7937 [9] culture procedures were used to identify *C. perfringens* in meat samples [10]. Each meat sample (weighing 10 g) was added to 10 mL of 0.1% peptone in water, stomached for 30 s to homogenise it, and then incubated anaerobically for 24 hours at 37°C (Remi, CO_2 Incubator, India). Following inoculation into 9 mL of cooked meat medium (HiMedia, Mumbai, India), the sample was heated at 75°C for 20 minutes to kill vegetative cells of competing microorganisms. Thereafter, the sample was incubated at 37°C for 24-48 hours using a gas pack under anaerobic conditions in a McIntosh anaerobic jar [11]. After being streaked onto tryptose sulfite cycloserine agar (TSC, HiMedia) with 5% egg yolk emulsion and rehydrated supplement components, the inoculated broth was incubated at 37°C for 24 h in anaerobic circumstances (Anaerobic Gas Pack, HiMedia, Mumbai, India). Colony morphology was used for presumptive identification; similarly, colonies that were black with a white halo were evaluated as positives.

Gram staining was used to identify suspicious colonies, after which they were chosen for biochemical confirmation using a variety of tests, such as the tests for motility, nitrate reduction, lecithinase, indole, hemolysis on blood agar, sugar fermentation (sucrose, glucose, and lactose), and gelatin liquefaction. All strains of *C. perfringens* were kept in 50% glycerol stocks and kept at -80°C before use [12].

Table-1 Detail interpretation criteria of antimicrobial discs

SN	Antibiotic discs	Concentrations (mcg)	Diameter of zone of inhibition (mm)		
			R	I	S
1	Amikacin (AK)	30	14	15-16	17
2	Cefixime (CFM)	5	15	16-18	19
3	Vancomycin (VA)	5	12	-	12
4	Moxifloxacin (MO)	5	20	21-23	24
5	Ciprofloxacin (CIP)	5	15	16-20	21
6	Tetracycline (TE)	30	14	15-18	19
7	Norfloxacin (NX)	10	12	13-16	17
8	Ofloxacin (OF)	2	14	15-17	18
9	Gentamicin (GEN)	30	19	20-22	25
10	Chloramphenicol (C)	30	12	13-17	18
11	Amoxicillin + clavulanic acid (AMC)	30-Oct	13	14-17	18
12	Cefalexin (CX)	30	14	15-17	18
13	Ceftazidime/Tazobactam (CAT)	30-Oct		17-24	
S- Sensitive, I - Intermediate and R - Resistant					

Antimicrobial susceptibility testing

Using the Clinical and Laboratory Standards Institute's guidelines, *C. perfringens* isolates were conducted antimicrobial resistance tests (CLSI, 2022). Based on their mode of action and data obtained from veterinary and medical professionals at the participating hospitals that are currently used in clinical therapy, the 13 antimicrobial disc antibiotics employed in this experiment [Table-1] were chosen. Following the recommendations of CLSI (2022), the antimicrobial discs used in this investigation were purchased from HiMedia Laboratories Ltd., Mumbai, and their quality was evaluated using ATCC 25923 Staphylococcus aureus. A loopful of pure culture from each test isolate was placed into a tube with 5 ml of Mueller Hinton Broth for an overnight incubation at 37°C with anaerobic conditions (MHB). For an overnight incubation at 37°C, the plates were inverted and kept in a McIntosh anaerobic jar with a gas pack (CLSI, 2022). Isolates were tested for sensitivity and resistance against amikacin (AK, 30 µg), vancomycin (VA, 5 µg), cefixime (CFM, 30 µg), norfloxacin (NX, 10 µg), moxifloxacin (MO, 5 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TE, 30 µg), Ceftazidime/Tazobactam (CAT, 30/10 µg), ofloxacin (OF, 2 µg), chloramphenicol (C, 30 µg), gentamicin (GEN, 30 µg) and cefalexin (CX, 30 µg),

Results and Discussion

Prevalence of *C. perfringens* in meat samples

In the current investigation, we researched into the prevalence of *C. perfringens* in meat from carabeef, chicken, mutton, and chevon. The antimicrobial resistance of the bacteria was assessed using samples that were procured at a wholesale market in Anand, Gujarat. The 200 meat samples resulted in the identification of 31 *C. perfringens* strains. Chicken meat (12/50, 24%) had the largest incidence, followed by chevon (8/50, 16%), carabeef (6/50, 12%), and mutton (5/50, 10%) [Fig-1]. The frequency and spread of *C. perfringens* in retail food samples have been the subject of numerous investigations, primarily in developed states. One of the few studies that quantify the prevalence of this bacterium in India is the one being presented here. Between 8% and 71% of the meat samples in Japan included *C. perfringens* [13].

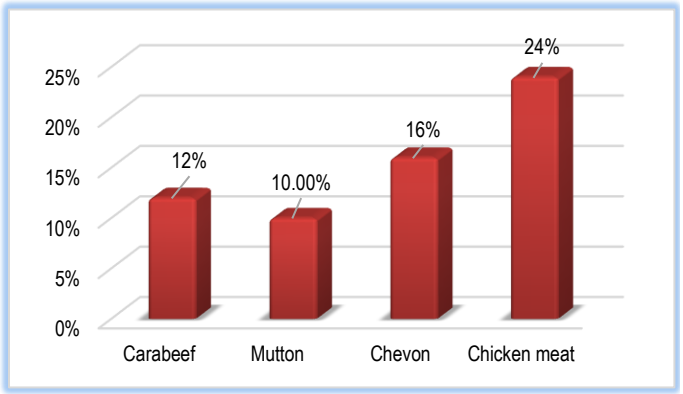

Fig-1 Column chart showing prevalence of *C. perfringens* in meat samples of various species

Our findings corroborated a previous study that detected a 17/77 (22%) prevalence of *C. perfringens* in chicken meat samples in Korea [14]. Our data showed that the prevalence of *C. perfringens* was highest in chicken meat samples compared to other forms of meat. *C. perfringens* was prevalent in samples of chicken meat from 67/155 (43%) studies conducted in Jordan [15]. It's interesting to note that a prior study found a significant incidence of *C. perfringens* in processed meat and the intestinal tract of poultry [16]. This is probably because *C. perfringens* colonies the chicken's gastrointestinal tract very early in life, even starting at the hatchery [17].

Our findings were greater than those of Singh *et al*. (2005)[18], Ghoneim and Hamza (2017) [19], and Birla *et al*. (2018) [20], who reported *C. perfringens* in carabeef sample isolation rates of 65.7%, 43.5%, and 28%, respectively. *C. perfringens* was found to be 16% common in chevon. Previous studies by Singh (2010) [21] and Birla *et al*. (2018) observed that chevon had prevalence's of *C. perfringens* of 58% and 20%, respectively. Yadav *et al*. (2017) [22] discovered a lower recovery rate of *C. perfringens* from chevon, namely 6%, which is lower than the current study. Lower than the current study, *C. perfringens* was found in 1.1% of the 92 lamb meat samples analysed in Australia and 8.0% of the 50 sheep meat samples tested in Pakistan. The existence of microorganisms in all types of meat samples supports earlier findings that bacteria are prevalent in nature, especially in the intestinal tracts of humans and animals, making the presence of pathogens in different diets impossible if meat is cooked improperly. The above study's outcomes were influenced by a few issues, including variations in analytical procedures, human and equipment hygiene, sample collection methods, and slaughterhouse technology.

Antimicrobial resistance of the *C. perfringens* isolates from meat samples

Antimicrobials have been utilized to boost animal growth and stop several contagious infections. Contrarily, their use has paradoxically boosted bacterial resistance to antibiotics [23]. All 31 *C. perfringens* collected isolates from various sources were tested against thirteen different antibacterial drugs used in therapeutic treatment to determine their in vitro antibiotic resistance patterns. Each isolate had a unique pattern of drug sensitivity and resistance. The most sensitive combination was amoxicillin/clavulanic acid (74%), followed by ceftazidime/tazobactam (71%), amikacin/cefixime (65%), vancomycin/ofloxacin (58%), norfloxacin/gentamicin (52%), chloramphenicol (45%), and ciprofloxacin (42%). Cephoxitin (61%) and moxifloxacin (52%), tetracycline (48%), vancomycin (42%), gentamicin (36%), and ofloxacin (32%), all showed resistance in meat samples [Fig-2].

Agarwal *et al*. (2009)[24], who observed sensitivity to ciprofloxacin (93.3%) and chloramphenicol (76.60%) during an in-vitro sensitivity test of 30 isolates to 11 antimicrobial drugs, which is greater than the current study, are among the Indian researchers who have reported comparable findings. A different study in Coimbatore, Tamil Nadu Skariyachan *et al*., (2010) [25] showed that *C. perfringens* isolates had substantially higher sensitivity to gentamicin (96.73%), tetracycline (93.47%), and vancomycin (92.39%), compared to the current study. The usage of antibiotics by animals and the poultry industry may be too responsible for this. In contrast to our work, Rahman *et al*. (2012) [26] found that *C. perfringens* isolated from animals and birds is more sensitive to the antibiotic's ciprofloxacin and norfloxacin.

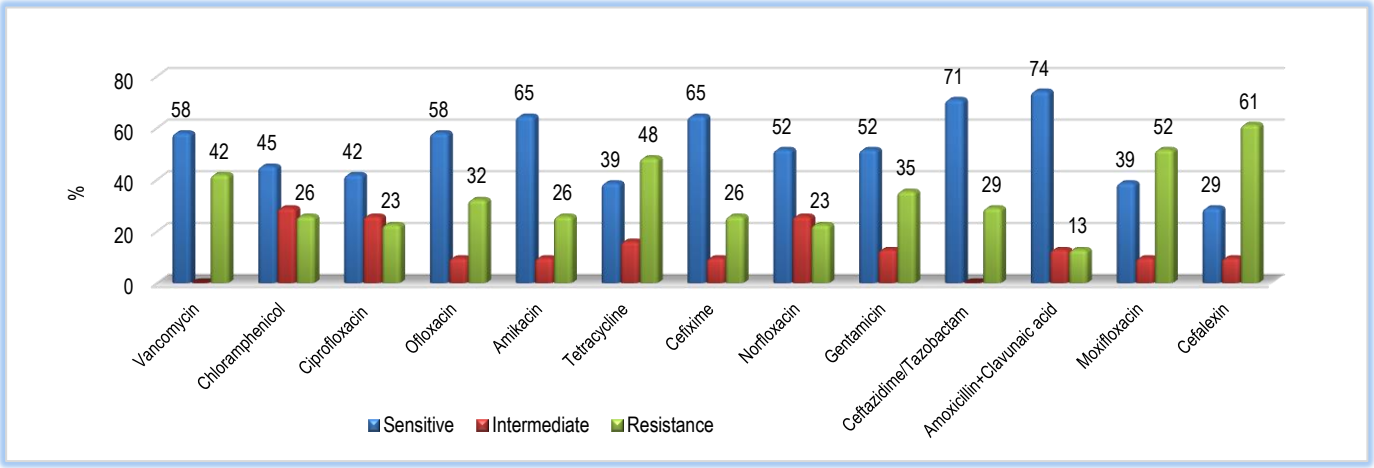

Fig-2 Antimicrobial susceptibility and resistance patterns of meat samples isolates

Most isolates from prior study that came from animals (94.46%) were found to be ciprofloxacin sensitive. Mehtaz *et al*. (2013) [27] observed ciprofloxacin (88.78%), ofloxacin (82.65%), and norfloxacin (80.61%) sensitivity in chicken and meat from clinically ill but seemingly healthy animals. Other antibiotic showed significant resistance, including norfloxacin (67%) and ciprofloxacin (58%) [28].

Tetracyclines and other antimicrobials are still used in many countries, including Switzerland, Denmark, Norway, Belgium, and Brazil, to treat *C. perfringens* infection in animals. According to earlier studies, *C. perfringens* isolates were reported to be highly resistant to tetracycline but vulnerable to β-lactam, nitroimidazoles, and carbapenems [29]. Tetracyclines, on the other hand, have been the most frequently used antibiotics since 2009, per a study of antibiotic use in Indian dairy farms [30]. Additionally, according to Hu *et al*. (2018), more than 93% of the *C. perfringens* tetracycline resistance was found in isolates from beef, chicken, duck, and pork meat. A prior investigation revealed that Clostridium spp. may carry tetracycline resistance genes that produce a cytoplasmic protein that protects ribosomes. Numerous investigations have demonstrated the potent inhibition of *C. perfringens* by β-lactam antibiotics was identified from beef, poultry, and pork meat that was obtained in Belgium, the United States, Scandinavia, and India [31-33].

Currently, multi-drug resistance in *C. perfringens* is common and/or emerging. The prevalence is rising and is a serious public health concern [34]. According to a recent study, most multidrug-resistant bacteria found in retail meats come from veterinary hospitals or farms where animals obtain antibiotics as feed or to treat diseases [35,36]. In the current investigation, 10 of the 31 isolates derived from distinct meat samples were multidrug resistant. It is essential to practise antimicrobial stewardship at the farm stage because genes encoding antimicrobial resistance can be passed across bacteria, which can be seen during the food production process.

Conclusion

However, because we did not look at *C. perfringens* spores in meat samples, the prevalence of *C. perfringens* reported in the present investigation might not accurately reflect the situation. In addition, the study's sample size was somewhat small, and the data we had on antimicrobial use on farms was insufficient. Accordingly, our work examined the *C. perfringens* antimicrobial resistance that was identified from domestic retail meats. Multiple-drug resistance bacteria may develop because of unrestrained antimicrobial use in animals. As a result, ongoing research on the antimicrobial resistance of *C. perfringens* isolates found in retail meats is required, as well as regular monitoring.

Application of research: Prevalence and Antimicrobial Susceptibility of *Clostridium perfringens* from various meat samples

Research Category: Veterinary Microbiology, Veterinary Epidemiology

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Study area / Sample Collection: Anand districts of Gujarat

Cultivar / Variety / Breed name: Nil

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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