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Investigation of Epidemiology and Assessment of Antimicrobial Resistance of *Clostridioides difficile* in Animal-Based Foods

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Abstract

In hospitalized patients, the prevalent nosocomial bacterium Clostridioides difficile causes diarrhea and gastrointestinal issues. The majority of C. difficile cases in the community are unconnected to antibiotic prescriptions or hospitalization, hence the dietary component has been highlighted as a vector of infection transmission. To look at the occurrence and antibiotic susceptibility of C. difficile isolated from raw meat and carcass surface swab samples, an existing survey was created. A total of 135 surface swab samples of raw beef and carcass were taken. Using a mix of biochemical methods and culture, C. difficile was isolated. The minimal inhibitory concentration (MIC) was devised in order to evaluate antibiotic resistance in isolates. There was evidence of C. difficile contamination in 4.57% of the samples analyzed. The pathogens were found in about 2.45% of raw meat samples and 3.77% of surface swabs from carcasses. There was evidence of resistance to tetracycline (75.67%), erythromycin (67.75%), metronidazole (37.55%), ciprofloxacin (42.65%), and clindamycin (55.43%). Meropenem and chloramphenicol had the lowest levels of resistance to the C. difficile bacteria (15.56% and 14.77%), respectively. C. difficile bacteria were shown to be lethal and antibiotic-resistant in surface swab samples taken from carcasses and raw meat. According to this study, food animals, particularly sheep and cattle, are carriers of C. difficile during the slaughter stage, which causes the carcasses in the slaughterhouse to be contaminated.

Keywords: Clostridioides difficile, epidemiology, antimicrobial resistance, meat

Introduction

Gastrointestinal symptoms brought on by the gram-positive, spore-forming bacteria Clostridium difficile (C. difficile) can range from moderate febrile diarrhea to violent colitis, septic shock, lethal megacolon, organ rupture, and even death (van Werkhoven et al., 2021). Among patients with nosocomial diarrhea, CDAD prevalence was 14.7%, and mortality was 7.9%, according to a recent meta-analysis of 51 studies. C. difficile infection (CDI) has recently become more common in both community and hospital settings (Feuerstadt et al., 2022). The increased prevalence of CDI in hospitalized patients has resulted in a global and hospital stay as a result of an overabundance of knowledge regarding the risks associated with overusing broad-spectrum antimicrobials and the implications of working to improve healthcare and environmental hygiene (Guh et al., 2020; Brajerova et al., 2022). Considering stomach acid production is the primary host defense mechanism against ingested C. difficile and its spores, broad-spectrum antibiotics and protons pump inhibitors (PPIs) disrupt healthy intestinal bacteria and because Clostridium react to different circumstances diarrhea to reoccur (CDAD). The most often cited risk factors include ageing, comorbidity, antibiotics use, knowledge of the hospital environment, and obesity (Schnizlein et al., 2022; Knight et al., 2019). Nonsteroidal anti-inflammatory drug use, poor vitamin D levels, and genetics are further risk factors (Knetsch et al., 2018; Weese et al., 2020). To determine the risk factors for CDI, studies have been carried out all over the world, but comparatively little study has been done in developing countries. By identifying and trying to manage the aforementioned risk factors, infection frequency and its related effects can be decreased. After receiving an effective dose, the anaerobe Gram-positive spore-bearing bacterium Clostridioides difficile (C. difficile) causes toxic megacolon, necrotizing enterocolitis, and diarrheal clinical symptoms. The possibility of death exists when the sickness is serious (Lim et al., 2020; Alves et al., 2022). There were 15,512 C. difficile cases in the US in 2017, according to reports. According to a meta-analysis released on September 30, 2021, C. difficile infections (CDIs) predominated in 12.40% of Asian countries (Rodriguez et al., 2016).

After getting an antibiotic prescription, toxic megacolon, necrotizing enterocolitis, and intestinal cramping symptoms are brought on by the anaerobe Gram-positive spore-bearing bacterium Clostridioides difficile (C. difficile). The possibility of death exists when the sickness is serious. There were 15,512 C. difficile infections in the US in 2017, according to reports. According to a meta-analysis released on September 30, 2021, infectious infections caused by C. difficile were prevalent in 12.40% of Asian countries (CDIs) (Kachrimanidou et al., 2019). Due to medication schedules, contaminated rivers and streams, and hospitalized patients, CDIs are more frequent. As C. difficile is spread by animal species and livestock corpses could become exposed to it prior to and following the slaughter process, there may be a chance that meat will be contaminated at retail. Bovine, swine, ovine, buffalo, caprine, camel, and chicken meat samples have all previously contained C. difficile, proving the significance of meat in the spread of bacteria (Bouttier et al., 2010). Some virulence and toxigenic components are necessary for CDI pathogenesis. The toxins enterotoxin (Toxin A) and cytotoxin (Toxin B), which are generated by the genes tcdA and tcdB, may directly affect host epithelial cells. Bacterial adherence to the intestinal epithelium was boosted by binary toxins with enzymatic (cdtA) and binding (cdtB) components. The main cause of C. difficile pathogenicity is these toxins. Several extremely virulent C. difficile ribotypes, including 077 and 027, are known to be able to produce spores and toxins (Pires et al., 2018).

These two ribotypes, as well as isolates from farm animals and dietary samples, are linked to severe CDI infections in humans. Antibiotics may lessen CDI symptoms while potentially hastening the progression of the condition. Enzymatic (cdtA) or binding (cdtB) binary toxins boosted bacterial adherence to the gut wall. The main cause of C. difficile pathogenicity is these toxins. It is believed that some extremely virulent C. difficile ribotypes can produce spores and toxins. Severe CDI cases in people, as well as those isolated from food animals and food samples, are linked to these two ribotypes (Rodriguez-Palacios et al., 2020; Abdel-Glil et al., 2018). Antibiotics can alleviate CDI signs while also possibly contributing to the disease's development. Furthermore, few studies on the C. difficile toxigenic gene profile, polymerase chain reaction (PCR)-ribotyping, and antibiotic susceptibility testing have been conducted in Pakistan. This appears to be the first study in Pakistan to look at toxigenic profiles, antibiotic resistance, PCR-ribotyping, and the prevalence of C. difficile infection in raw meat and carcass surface swab samples, among other things.

Materials and Methodology

Study Area

In Pakistan, a tertiary care hospital served as the setting for this case-control study. About 220 patients with CDAD were enrolled in the trial (n=220) between June 2020 and March 2021 using a consecutive simple non-probability sampling method. After receiving informed consent, patients were added to the study. Before enrolling patients, Liaquat University of Medical and Health Sciences' clearance for the ethical review process was requested (LUMHS/2020/ERC-16). Stool enzyme immunoassay results and clinical symptoms (diarrhea) were used to diagnose CDAD (Kordus et al., 2022). As a control group, 220 additional outpatient patients without a CDAD diagnosis were enrolled.

Patients' comprehensive histories were recorded in a self-structured questionnaire, including the use of antimicrobials, H2RA, PPI, and previous history of CDI, and hospitalization within the previous 30 days. The patients' height and weight were calculated and recorded in a questionnaire. A self-structured survey also collected information on comorbidities such as diabetes, chronic renal failure, hypertension, and cancer.

Samples

A total of 475 samples, including surface swabs from the carcasses of cattle (n = 75), sheep (n = 70), and goats (n = 70), as well as raw meat from beef, lamb, and goats (n = 70), were randomly selected from the animals that were transported to slaughterhouses in Rawalpindi, Pakistan. The raw beef sample was intended to be taken from a tight muscle. For that, about 100 g of tight muscle were removed using forceps and placed in sterile plastic bags. The laboratory quickly (within 2 hours) and in a refrigerated state received the gathered samples. The transportation and processing of the sample were finished two hours after the sample was collected. Following bleeding, skinning, eviscerating, and washing, carcass samples were taken from the tight muscle using the swabbing method. First, buffered peptone water containing 0.5% cysteine was used to wet the swab samples (Oxoid, UK).

C. difficile Isolation and Identification

A sterile container was filled with 25 ml of phosphate-buffered saline and 25 g of collected raw beef samples. Then, add 9 mL of C. difficile broth, which contains defibrinated sheep blood (5% (v/v)), fructose (6.0 g/l), sodium chloride (2.0 g/l), disodium hydrogen phosphate (5.0 g/l), and protease peptone (40 g/l). After 10 to 15 days of incubation at 37°C, shake the mixture (2 mL of the incubated broth was combined with 2 mL of ethanol) for 50 minutes. After that centrifuge this mixture at 3800 g for ten minutes. After that, the plates were streaked with sediment and underwent a 48-hour anaerobic incubation at 37°C. On Oxoid CM0131 (UK) tryptone soya agar (TSA), tree colonies were subculture, and they were then analyzed using common biochemical and microbiological techniques (Martínez-Meléndez et al., 2022).

Antibiotic Resistance in C. difficile.

There were eleven different antimicrobial medications used, including erythromycin, clindamycin, levofloxacin, ciprofloxacin, moxifloxacin, tetracycline, meropenem, chloramphenicol, and vancomycin, among others. For this, Brucella agar media (Oxoid, UK) was employed. The media was aided with 5% broth and incubated sheep blood, 30 µg/L chlorphenamine and 1 mg/L vitamin K1 (Oxoid, UK) (Herbert et al., 2022).

Statistical analysis

The Statistical Software for Social Sciences was used for statistical analysis (SPSS version 23.0).

Results

C. difficile Frequency in Tested Samples

The frequency of C. difficile is seen in Table 1 for the surface carcass swab samples and raw meat samples. About 18 of the 475 raw meat and surface carcass swab samples analyzed had C. difficile. In surface swab samples and 2.91% of raw meat samples from diverse animal species, C. difficile was discovered. Surface swab samples and raw meat had very different C. difficile prevalence (P 0.05). Sheep meat was the raw meat sample where C. difficile was found most frequently (5%). Furthermore, sheep carcasses had the highest frequency of C. difficile (7.50%) among surface swab samples. The incidence of C. difficile and sample type varied significantly ($P<0.05$). Diarrhea in outpatients and people who have not interacted with medical institutions is increasingly being linked to CDI.

In the current investigation, C. difficile spores were discovered in raw meat samples (2.91%) and carcass surface swab samples (3.71%). Our results demonstrated a lower prevalence of C. difficile in carcass surface and swabs raw meat samples when compared to earlier research. However, compared to earlier research, the prevalence rate revealed in the current study was higher. In raw meat samples of beef, pork, and chicken, C. difficile was present at levels of 16.40%, 7.30%, and 6.70%, respectively, according to a Korean investigation. In a different study from Pakistan, the prevalence of C. difficile was 9%, 3.30%, 1.70%, 0.94%, and 0.90%, respectively, in raw buffalo, goat, cow, beef, and sheep meat samples. In raw meat samples of sheep and cow acquired in Saudi Arabia, C. difficile was present in 3.5% and 1%, respectively.

Table 1: *C. difficile* prevalence amongst the examined raw meat and surface carcass swab samples.

Types of samples	N. collected samples	N (%). Positive for *C. difficile*
Raw cattle meat	70	2 (4.50)
Raw sheep meat	70	5 (7)

Raw goat meat	70	3 (3.25)
Total	210	10 (2.91)
Cattle carcass surface swab	75	6 (7.52)
Sheep carcass surface swab	70	8 (9.50)
Goat carcass surface swab	70	7 (5.50)
Total	215	21 (8.47)
Total	475	31 (6.71)

Clostridium difficile Pattern of Antibiotic Resistance

Based on C. difficile MIC levels, Table 2 displays antibiotic resistance. The highest resistance rates to tetracycline, erythromycin, metronidazole, ciprofloxacin, and clindamycin were found in C. difficile isolates from surface carcass swab and raw meat samples, respectively. The lowest rates of resistance were to meropenem and chloramphenicol. Antibiotic resistance was higher in C. difficile isolates from raw meat samples than it was in isolates from carcass surface swab samples ($P<0.05$). Additionally, statistically significant differences between the sample type and C. difficile resistance rate were discovered ($P<0.05$). Some of the most widely used CDI therapies, such as erythromycin, tetracycline, ciprofloxacin, metronidazole, and clindamycin, were less effective against C. difficile strains.

Table 2: C. difficile antibiotic resistance

	C. difficile antibiotic resistance in samples (%)					
Antimicrobial Agents	**Raw cattle meat**	**Raw sheep meat**	**Raw goat meat**	**Cattle carcass surface swab**	**Sheep carcass surface swab**	**Goat carcass surface swab**
Tetracycline (10µg)	97	99	92	70	77	66
Erythromycin (10µg)	97	99	92	70	77	67
Metronidazole (300µg)	90	65	45	70	97	44
Clindamycin (5µg)	41	70	45	45	55	33
Meropenem (30µg)	25	57	0	25	27	10
Chloramphenicol (30µg)	22	27	0	15	55	10

Discussion

Those who were previously believed to be at minimal risk, such as children and those who weren't exposed to antibiotics, have been shown to have community-associated illnesses. Food has been mentioned as a potential C. difficile source in the area, although there is not enough data to confirm or deny this. C. difficile infects food animals' stomachs and causes diarrhea. In a number of countries, including Australia, the United States, and Europe, C. difficile has been discovered in retail products meant for human consumption (Janezic et al., 2012). Assessing the likelihood of food contamination transfer and the significance of animal-human interaction in C. difficile empirical studies will be made easier with a better understanding of the correlation between the human and animal strains of the bacterium. In terms of the prevalence of C. difficile in food, there are, nonetheless, necessary attributes gaps (Weese et al., 2010). In the current investigation, C. difficile spores were discovered in 2.91% of raw meat records and 3.71% of carcass surface swab samples. Our results demonstrated a reduced prevalence of C. difficile in raw meat samples and carcass surface swabs when compared to earlier studies. Nonetheless, the new study discovered a higher prevalence rate when compared to other studies (Banawas et al., 2018). C. difficile was found in raw meat samples of beef, pig, and chicken at levels of 16.40%, 7.30%, and 6.70%, respectively, according to a Korean investigation. In a different Pakistani investigation, the prevalence of C. difficile was 9%, 3.30%, 1.70%, 0.94%, and 0.90%, respectively, in raw buffalo, goat, beef, cow, and sheep meat samples. C. difficile was found in 3.5% and 1% of raw meat samples from sheep and cow that were collected in Saudi Arabia, respectively (Sholeh et al., 2020). C. difficile was discovered in surface samples from corpses of sheep and cattle at frequencies of 25.30% and 33.60%, respectively, in a Turkish investigation. Belgian carcasses of cattle and pigs were contaminated with C. difficile at rates of 7.90% and 7%, respectively. Yet in Australia, 25.30% of cow carcasses contained C. difficile. Between 0% to 43% of retail beef has been identified as having C. difficile bacteria. However, in raw meat samples, especially those other than cattle, C. difficile couldn't differentiate between other animal species. Variations in prevalence could be caused by changes in geographical area, isolation methodology, and antibacterial drugs dosage levels (Saha et al., 2019).

Our results showed a greater C. difficile infection rate in raw sheep meat and surface swab samples from sheep corpses compared to other animal species. Results from other counters support our finding that samples of harvested sheep meat contained higher concentrations of C. difficile. A higher incidence of C. difficile, however, was discovered in meat samples and cow carcasses in some studies. Why sophisticated C. difficile is considerably more prevalent in sheep than in cattle and goats is unknown. Goat samples had the lowest contamination incidence, which was in line with the findings of Rahimi et al. and Bakri. The uplands are where goats usually reside, where they are free to graze without even being troubled by people. This may account for the reduced C. difficile prevalence in samples from goats. Surface swab samples from carcasses contained higher amounts of C. difficile than raw meat did (Janezic et al., 2012). This discovery has been most likely brought about by handling and interaction with the contaminated environment of the slaughterhouse, which contaminated the surface of the animal carcass. In conclusion, the primary sources of C. difficile in the samples examined are animal gastrointestinal tracts and the possibility for cross-contamination from meat factory procedures to animal carcasses (De Boer et al., 2011). Antibiotic resistance could be explained by a number of factors, including unapproved and reckless delivery of drugs, antibacterial drugs and antiseptics overdose, and antibiotic self-treatment. Antibiotic-resistant bacteria have been linked to contaminated personnel and the slaughterhouse environment (Weese et al., 2010). In experiments carried out in China, Saudi Arabia, and Pakistan, C. difficile strains were discovered to be resistant to metronidazole, tetracycline, erythromycin, clindamycin, and ciprofloxacin. Further trials conducted in Korea, Manitoba, Turkey, and Italy indicated that metronidazole and rifampicin were particularly efficient against C. difficile strains. C. difficile bacteria were completely destroyed by the antibiotics vancomycin, tetracycline, metronidazole, clindamycin, and moxifloxacin when they were isolated from raw meat samples of several animal species. Han et al. discovered that C. difficile bacteria isolated from packaged food were 100% resistant to metronidazole, 100% resistant to vancomycin, 100% resistant to clindamycin, 100% resistant to erythromycin, and 36.60% high resistance to cefotaxime. Tsuchiya et al. discovered that C. difficile had 76.40%, 63.60%, 22.70%, 40.90%, 9.10%, and 13.60% antibiotic resistance to clindamycin, ceftriaxone, ceftazidime, tetracycline, metronidazole, and vancomycin, respectively (Rodriguez-Palacios et al., 2020; Abdel-Glil et al., 2018). The prevalence of resistance to ampicillin, ciprofloxacin, chloramphenicol, gentamicin, clindamycin, doxycycline, metronidazole, erythromycin, nalidixic acid, tetracycline, and vancomycin was reported by Rahimi et al. to be 53.75%, 0%, 76.92%, 92.31%, 0%, 61.54%, 100%, 0%, 100%. The availability or lack of antibiotics, stringent antibiotic prescribing guidelines, and medical professionals' and veterinarians' perspectives on antibiotic use are all factors that have undoubtedly contributed to the increases in antibiotic resistance that have been observed in various studies. To discover antibiotic resistance in C. difficile strains, various methods (MIC and simple disc diffusion) were employed. Meropenem and chloramphenicol were poorly resistant. The delivery of chloramphenicol under forbidden circumstances and the use of meropenem in hospitals are to blame.

Conclusions

Overall, toxic and antibiotic-resistant isolates of C. difficile that were isolated using surface swab samples from meat and carcass from cattle, sheep, and goats. The most contaminated samples came from carcasses and sheep meat, which had high contamination rates. C. difficile isolates were found to have antibiotic resistance to ciprofloxacin, metronidazole, erythromycin, tetracycline, and clindamycin. Furthermore, it was found that surface swab samples from raw meat and carcasses contributed to the spread of the dangerous, poisonous, and antibiotic-resistant C. difficile to the general populace. At the slaughterhouse, animals are C. difficile carriers, contaminating the meat and carcass.

References

Abdel-Glil MY, Thomas P, Schmoock G, Abou-El-Azm K, Wieler LH, Neubauer H, Seyboldt C (2018). Presence of Clostridium difficile in poultry and poultry meat in Egypt. Anaerobe, 1;51:21-5.

Alves F, Nunes A, Castro R, Sequeira A, Moreira O, Matias R, Rodrigues JC, Silveira L, Gomes JP, Oleastro M (2022). Assessment of the transmission dynamics of Clostridioides difficile in a farm environment reveals the presence of a new toxigenic strain connected to swine production. Frontiers in Microbiology,1230.

Banawas SS (2018). Clostridium difficile infections: a global overview of drug sensitivity and resistance mechanisms. BioMed research international, 20-18.

Bouttier S, Barc MC, Felix B, Lambert S, Collignon A, Barbut F (2010). Clostridium difficile in ground meat, France. Emerging Infectious Diseases, 4: 733–735,

Brajerova M, Zikova J, Krutova M (2022). Clostridioides difficile epidemiology in the Middle and the Far East. Anaerobe, 1;74:102542.

De Boer E, Zwartkruis-Nahuis A, Heuvelink AE, Harmanus C, Kuijper EJ (2011). Prevalence of Clostridium difficile in retailed meat in the Netherlands. International journal of food microbiology, 5;144(3):561-4.

Feuerstadt P, Nelson WW, Drozd EM, Dreyfus J, Dahdal DN, Wong AC, Mohammadi I, Teigland C, Amin A (2022). Mortality, health care use, and costs of Clostridioides difficile infections in older adults. Journal of the American Medical Directors Association, 1;23(10):1721-8.

Guh AY, Mu Y, Winston LG, Johnston H, Olson D, Farley MM, Wilson LE, Holzbauer SM, Phipps EC, Dumyati GK, Beldavs ZG (2020). Trends in US burden of Clostridioides difficile infection and outcomes. New England Journal of Medicine, 2;382(14):1320-30.

Herbert R, Hatcher J, Jauneikaite E, Gharbi M, d'Arc S, Obaray N, Rickards T, Rebec M, Blandy O, Hope R, Thomas A (2019). Two-year analysis of Clostridium difficile ribotypes associated with increased severity. Journal of Hospital Infection, 1;103(4):388-94.

Janezic S, Ocepek M, Zidaric V, Rupnik M. Clostridium difficile genotypes other than ribotype 078 that are prevalent among human, animal and environmental isolates. BMC microbiology. 2012 Dec;12(1):1-8.

Kachrimanidou M, Tzika E, Filioussis G (2019). Clostridioides (Clostridium) difficile in food-producing animals, horses and household pets: a comprehensive review. Microorganisms, 9;7(12):667.

Knetsch CW, Kumar N, Forster SC, Connor TR, Browne HP, Harmanus C, Sanders IM, Harris SR, Turner L, Morris T, Perry M (2018). Zoonotic transfer of Clostridium difficile harboring antimicrobial resistance between farm animals and humans. Journal of clinical microbiology, 56(3):e01384-17.

Knight DR, Riley TV (2019). Genomic delineation of zoonotic origins of Clostridium difficile. Frontiers in public health, 20;7:164.

Kordus SL, Thomas AK, Lacy DB (2022). Clostridioides difficile toxins: mechanisms of action and antitoxin therapeutics. Nature Reviews Microbiology, 20(5):285-98.

Lim SC, Knight DR, Riley TV (2020). Clostridium difficile and one health. Clinical Microbiology and Infection, 1;26(7):857-63.

Martínez-Meléndez A, Cruz-López F, Morfin-Otero R, Maldonado-Garza HJ, Garza-González E (2022). An update on Clostridioides difficile binary toxin. Toxins, 27;14(5):305.

Pires RN, Caurio CF, Saldanha GZ, Martins AF, Pasqualotto AC (2018). Clostridium difficile contamination in retail meat products in Brazil. Brazilian Journal of Infectious Diseases, 22:345-6.
Rodriguez C, Taminiau B, Van Broeck J, Delmée M, Daube G (2016). Clostridium difficile in food and animals: a comprehensive review. Advances in Microbiology, Infectious Diseases and Public Health, 4:65-92.
Rodriguez-Palacios A, Mo KQ, Shah BU, Msuya J, Bijedic N, Deshpande A, Ilic S (2020). Global and historical distribution of Clostridioides difficile in the human diet (1981–2019): systematic review and meta-analysis of 21886 samples reveal sources of heterogeneity, high-risk foods, and unexpected higher prevalence toward the tropic. Frontiers in Medicine, 27;7:9.
Saha S, Kapoor S, Tariq R, Schuetz AN, Tosh PK, Pardi DS, Khanna S (2019). Increasing antibiotic resistance in Clostridioides difficile: A systematic review and meta-analysis. Anaerobe, 1;58:35-46.
Schnizlein MK, Young VB (2022). Capturing the environment of the Clostridioides difficile infection cycle. Nature Reviews Gastroenterology & Hepatology,19(8):508-20.
Sholeh M, Krutova M, Forouzesh M, Mironov S, Sadeghifard N, Molaeipour L, Maleki A, Kouhsari E (2020). Antimicrobial resistance in Clostridioides (Clostridium) difficile derived from humans: a systematic review and meta-analysis. Antimicrobial Resistance & Infection Control, 9:1-1.
van Werkhoven CH, Ducher A, Berkell M, Mysara M, Lammens C, Torre-Cisneros J, Rodríguez-Baño J, Herghea D, Cornely OA, Biehl LM, Bernard L (2021). Incidence and predictive biomarkers of Clostridioides difficile infection in hospitalized patients receiving broad-spectrum antibiotics. Nature communications, 14;12(1):2240.
Weese JS (2010). Clostridium difficile in food—innocent bystander or serious threat?. Clinical Microbiology and Infection, 1;16(1):3-10.
Weese JS (2020). Clostridium (Clostridioides) difficile in animals. Journal of veterinary diagnostic investigation, 32(2):213-21.