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Research Paper

ANTIMICROBIAL PROFILE OF CLOSTRIDIUM PERFRINGENS ISOLATES FROM BUFFEN, CHEVON, CHICKEN AND FISH MEAT

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To observe the antimicrobial profile of *Clostridium perfringens* isolates in different meat samples against 16 antibiotics. A total of 200 meat samples (50 each of buffen, chevon, chicken and fish meat) collected from retail outlets in Jabalpur city. Antimicrobial profile for isolates of *C. perfringens* was performed by disc diffusion method. Overall incidence of *C. perfringens* in meat sample was 23% with buffen-28%, fish meat-24% and chevon and chicken each-20%. Results revealed that most of the isolates were sensitive to amoxicillin and ofloxacin, whereas maximum isolates were found to be resistant against amikacin and co-trimoxazole. The resistance to the antimicrobials is an alarming sign because these antibiotics are broad spectrum and are commonly used for treatment of the diseases caused by the pathogen. It is now a global problem and is continuously increasing due to injudicious and indiscriminate use of antimicrobials in therapeutic management.

Keywords: Clostridium perfringens, Microbial profile, Meat samples, Therapeutic management

INTRODUCTION

C. perfringens is a non-motile, encapsulated, short and thick bacillus with blunt end and sub-terminal ovoid spores. Strains are divided in five toxicological types (A to E) based on four major toxins (α, β, ε, and ι). *C. perfringens* is associated with traumatic wound infections, anaerobic cellulitis, fasciitis and anaerobic myonecrosis associated with suppurative deep tissue infections and gas gangrene. Type A and C are the only strains associated with human gastroenteritis. *C. perfringens* is the third most common cause of food borne illness.

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C. perfringens is ubiquitous throughout the natural environment, commonly encountered in soil, foods (frequently in raw meat and poultry), dust and intestinal tracts of humans (10% to 30% of adults) and domestic animals (30% to 60% in beef meat, 30% to 50% in pork and 40% to 80% in poultry). It has predilection for protein rich foods particularly meat and their products. The food poisoning incidences occurred in institutions, cafeterias or banquets, where food is prepared in large quantity and kept for longer periods which give chance to vegetative cells to multiply. Food poisoning generally occurs due to the ingestion of approximately 10^5 to 10^7 viable vegetative cells/g of food. Incubation period is between 6-24 hrs (commonly 10-12 hrs) after ingestion of contaminated food and is spontaneously resolved within 12 to 24 hrs. The illness is characterized by a sudden onset of watery diarrhea and moderate to severe, crampy, mid epigastric pain. Vomiting and fever are uncommon. While everyone appears to be susceptible to *C. perfringens* food poisoning, the illness tends to be more severe in elderly or debilitated individuals. In the elderly, ill or debilitated individuals, serious complications have led to death (Miwa *et al.*, 1999; Murray *et al.*, 2003; Ryan and Ray, 2004; Orenga *et al.*, 2009; and Borah *et al.*, 2011).

Identification of enterotoxin in food is not successful as most of the enterotoxins are produced in the human gut after ingestion of food where *C. perfringens* are allowed to grow in large numbers. Because the fecal flora of healthy persons frequently includes *C. perfringens*, counts of at least one million *C. perfringens* spores per gram of feces obtained within 48 hrs of onset of illness are required to support the diagnosis in ill persons (*Clostridium Perfringens* Manual, 2015). So, the aim of the present study was to determine the incidence, molecular characterization and antimicrobial profile of *C. perfringens* in meat samples.

MATERIAL AND METHODS

Total 200 samples (each of 50 raw buffen, 50 raw chevon, 50 raw chicken and 50 fish meat) were collected from different retail outlets in and around Jabalpur city. Sample was collected in properly sterilized polythene bags and transfer to laboratory in chilled condition for bacteriological examination. All samples were processed for isolation within 12 hrs of arrival in the laboratory.

Isolation was done from the samples of meat according to Agarwal *et al*. (2003) with certain modifications One ml of diluted sample (1:10) was taken directly into 9 ml alternative thioglycollate broth and incubated. For isolation of , an agar over lay technique in petriplate was done using Tryptose Sulphite Cycloserine (TSC) agar containing egg yolk emulsion. Inoculum (0.1 ml) was spread on TSC agar containing egg yolk emulsion. After 5 min, plate was overlayed with 10 ml TSC agar without egg yolk emulsion. After solidification, the plate was incubated anaerobically overnight at 37 °C. Presumptive black colonies of *C. perfringens* was taken in alternative thioglycollate broth and incubated at 37 °C for 18-24 hrs and tested biochemically (Rhodehamel and Harmon, 1998).

Antibiotic sensitivity test for *C. perfringens* isolates against 16 antimicrobials was performed by disc diffusion method on Muller Hinton agar (MH) with certain modification (Clinical and Laboratory Standards Institute, 2013). The isolates were grown in alternative thioglycollate broth for 4-6 hrs at 37 °C. Spreading of culture was done across the entire agar surface and agar petriplate was kept at room temperature for 30 min to allow the inoculums to be absorbed on the surface. Antibiotic sensitivity octadisc (Hi-Media) was placed with the help of sterile forceps on to the plates. The plates were incubated in anaerobic condition at 37 °C. The results were interpreted according to the instructions of the manufacturer as given in Table 1.

RESULTS

A total of 200 samples (50 each of buffen, chevon, chicken and fish meat) were examined for isolation of *C. perfringens*. The results of the isolation study indicated that the 177 samples (buffen-44, chevon-42, chicken-44 and fish-47) showed presence of sulfide producing organisms. The overall incidence of *C. perfringens* was 23%. The highest incidence of *C. perfringens* was found in buffen 14(28%), followed by fish meat 12(24%) and chevon and chicken 10(20% each).

C. perfringens isolates were found sensitive to ofloxacin and amoxicillin (82.60%), cefotaxime (65.21%), chloramphenicol (54.34%) and resistant to amikacin (89.13%), co-trimoxazole (69.56%), lincomycin (67.39%) and ceftazidime (63.04%) as depicted in Table 2. Sample wise antimicrobial profile study revealed that ofloxacin was found sensitive to 92.85% isolates from buffen followed by fish (91.66%) and chevon and chicken (70% each). Amoxicillin showed sensitivity to 91.66% isolates from fish meat and

Table 1: Antibiotic Disc, Their Concentration and Interpretation

S. No.	Antibiotic Disc Used	Concentration (mcg)	Interpretation		
			R	I	S
1	Amoxicillin (AMX)	10	18	19 - 20	22
2	Tetracycline (T)	30	14	15 - 18	19
3	Co-trimoxazole (COT)	25	10	11 - 15	16
4	Ciprofloxacin (Cf)	5	15	16 - 20	21
5	Gentamicin (G)	10	12	13 - 14	15
6	Erythromycin (E)	15	15	16 - 20	21
7	Chloramphenicol (C)	30	12	13 - 17	18
8	Cefalexin (CN)	30	14	15 - 21	22
9	Ceftrixone (CTR)	30	13	14 - 20	21
10	Ceftazidime (CAZ)	30	14	15 - 17	18
11	Cefotaxime (CTX)	30	14	15 - 22	23
12	Lincomycin (L)	2	15	17 - 20	21
13	Netilmicin (NET)	30	12	13 - 14	15
14	Oflaxacin (OF)	2	12	13 - 15	16
15	Vancomycin (VA)	30	14	15 - 16	17
16	Amikacin (AK)	30	14	15 - 16	17

Note: R - Resistant, I - Intermediate, S - Sensitive.

Table 2: Antimicrobial Profile of C. perfringens Isolates from Meat

S. No.	Name of Antimicrobials	Number of Isolates Screened	Antimicrobial Profile of *C. perfringens* Isolates		
			Resistance	Intermediate	Sensitive
1	Amoxicillin (10 mcg)	46	3 (6.50%)	5 (10.86%)	38 (82.60%)
2	Tetracycline (30 mcg)	46	23 (50.00%)	6 (13.04%)	17 (36.95%)
3	Co-trimoxazole (25 mcg)	46	32 (69.56%)	10 (21.73%)	4 (8.69%)
4	Ciprofloxacin (5 mcg)	46	1 (2.17%)	22 (47.82%)	23 (50.00%)
5	Gentamicin (10 mcg)	46	19 (41.30%)	14 (30.43%)	13 (28.26%)
6	Erythromycin (15 mcg)	46	24 (52.17%)	11 (23.91%)	11 (23.91%)
7	Chloramphenicol (30 mcg)	46	10 (21.73%)	11 (23.91%)	25 (54.34%)
8	Cefalexin (30 mcg)	46	22 (52.17%)	22 (47.82%)	0 0.00
9	Ceftrixone (30 mcg)	46	8 (17.39%)	17 (36.95%)	21 (45.65%)
10	Ceftazidime (30 mcg)	46	29 (63.04%)	6 (13.04%)	11 (23.91%)
11	Cefotaxime (30 mcg)	46	9 (19.56%)	7 (15.21%)	30 (65.21%)
12	Lincomycin (2 mcg)	46	31 (67.39%)	9 (19.56%)	6 (13.04%)
13	Netamicin (30 mcg)	46	25 (54.34%)	14 (30.43%)	7 (15.21%)
14	Oflaxacin (2 mcg)	46	2 (4.34%)	6 (13.04%)	38 (82.60%)
15	Vancomycin (30 mcg)	46	4 (8.69%)	8 (17.39%)	34 (73.91%)
16	Amikacin (30 mcg)	46	41 (89.13%)	3 (6.52%)	2 (4.34%)

90.00% to chicken isolates. Cefotaxime was sensitive to 70% and 64.28% isolates from chevon and buffen, respectively. Amikacin displayed 100% resistant towards isolates from buffen, followed by chevon (92.85%) and chicken (80%).

DISCUSSION

Clostridium perfringens is one of the most common food borne organisms due to its ubiquitous nature. *C. perfringens* is a Gram positive, anaerobic, spore-forming, non-motile mesophilic rod. The pathogen leads to different forms of enterotoxaemia in animals and gas gangrene in animals and man. Based on their ability to produce certain exotoxins, five types are recognized: type A, B, C, D and E. Another important exotoxin is enterotoxin, which is mainly implicated in food borne incidences. Thus, the present study is aiming toward study of incidence of *C. perfringens* in different animal meat and their characterization on the basis of virulence and antimicrobial profile.

The study on 200 meat samples (50 each of buffen, chevon, chicken and fish meat) revealed the overall incidence of 23% of *C. perfringens*. The highest occurrence was found in buffen (28%), followed by fish meat (24%), chevon (20%) and chicken (20%). The presence of microorganisms in all types of meat samples validates the earlier findings that the bacteria are widespread in nature including the intestinal tract of humans and animals, which makes the pathogen presence in different food inevitable. Previous examinations also reported the occurrence of *C. perfringens* in various domestic animals viz. 30% to 60% in beef meat, 30% to 50% in pork and 40% to 80% in poultry (Ryan and Ray, 2004).

Difference in incidence have been reported by various workers in our country viz. Singh *et al.* (2006) during examination of *C. perfringens* in 211 meat samples in Bareilly, observed 91.40% incidence in goat, followed by poultry (70.40%) and buffalo (65.70%). Similarly, in another study, Singh (2010) studied the occurrence of *C. perfringens* in 461 different foods samples and found an overall occurrence of 57.70% with highest incidence in poultry (94.41%), followed by goat (58.00%) and fried chicken (16.66%) among meat sample. An investigation in Tamil Nadu from intestinal contents of 267 fresh water fishes revealed the prevalence of *C. perfringens* type A in 49 (18.35%) samples (Das and Jain, 2012). Likewise, examination of 400 food samples in Guwahati, displayed 33 food samples were positive for *C. perfringens* (Gurmu *et al.*, 2013). A high occurrence investigation (81.69%) was carried out in Bangalore, Karnataka by Prabhu *et al.* (2013) wherein, 71 chicken meat samples from retail outlets were observed. Yadav *et al.* (2016) examined 102 samples of fish and their products in Kolkata and found 24 (23.52%) samples to be *C. perfringens* positive.

C. perfringens isolates were found sensitive to ofloxacin and amoxicillin (82.60%), cefotaxime (65.21%), chloramphenicol (54.34%) and resistant to amikacin (89.13%), co-trimoxazole (69.56%), lincomycin (67.39%) and ceftazidime (63.04%). Similar findings have been reported in India by several workers viz. Agarwal *et al*. (2009) during an *in-vitro* sensitivity test of 30 isolates to 11 antimicrobial agents observed sensitivity to fluroquinolones like ciprofloxacin (93.30%) and enrofloxacin (86.60%), moderate sensitivity to chloramphenicol (76.60%), oxytetracycline (70%) and co-trimoxazole (63.30%). The organisms were 100% resistance to streptomycin and neomycin. Likewise, another study in Coimbatore, Tamilnadu recorded higher sensitivity for isolates of *C. perfringens* against ampicillin (100%), gentamicin (96.73%), tetracycline (93.47%) and vancomycin (92.39%) and least sensitivity for amphotericin-B (8.69%) (Skariyachan *et al.*, 2010). Another report from Kolkata, showed highest sensitivity to enrofloxacin (89.47%) and least to streptomycin (15.78%) (Das *et al.*, 2014). Likewise, Yadav *et al.* (2016) observed that 76.47% isolates of *C. perfringens* showed multidrug resistance phenomenon. Besides this, they recorded highest resistance against co-trimoxazole (88%), followed by ceftriaxone (87%), cefazidime (53%), tetracycline (44%), norfloxacin (21%) and amikacin (11%), while all the isolates were found to be sensitive to ciprofloxacin and amoxicillin/clavulinic acid.

Investigation in abroad by Gad *et al*. (2011) showed that *C. perfringens* isolates from turkey meat samples were susceptible to â - lactam antibiotics penicillin, amoxicillin and oxacillin, enrofloxacin combination of lincomycin and spectinomycin and trimethoprim/ sulfamethoxazole. The isolates were resistant to spectinomycin (74%) and neomycin (94%). All isolates were resistant to colistin. Slavic *et al.* [29] found that all the isolates of *C. perfringens* from fecal samples were resistant to multiple antibiotics. All 30 (100%) isolates were sensitive to penicillin and tetracycline, 18 (94.70%) to erythromycin, 17 (89.50%) to gentamicin, 16 (84.20%) to amoxicillin and 15 (78.90%) to ciprofloxacin and 13 (68.40%) to chloramphenicol. Mehtaz *et al.* (2013) recorded sensitivity to ciprofloxacin (88.78%), ofloxacin (82.65%), norfloxacin (80.61%) and enrofloxacin (78.57%), while least sensitivity to streptomycin (7.14%) in isolates from apparently healthy, clinically affected animals, chicken and meat and milk. An investigation in Lahore (Pakistan), revealed that isolates of *C. perfringens* from meat sample were susceptible to chloramphenicol, ciprofloxacin, metronidazole and ceftriaxone. All other drugs were relatively less effective with a least activity of amoxicillin (Khan *et al.*, 2014). A study in dairy products dispayed that isolates were sensitive to ofloxacin, ampicillin-sulbactam and norfloxacin (100%), vancomycin, tetracycline, metronidazole and amoxicillin-clavulinic acid (83.30%) and clindamycin (66.70%). The majority of the isolates were resistant to cephalothin (100%), sulphamethoxazole-trimethoprim (83.30%), oxacillin and chloramphenicol (66.70%) (Tawab *et al.*, 2016).

A study in Egypt, revealed high antibiotic resistance in the isolates of *C. perfringens* from chicken to gentamicin, streptomycin, oxolinic acid, lincomycin, erythromycin and spiramycin to all isolates. The resistance to other antibiotics was also observed high viz. rifampicin (34%), chloramphenicol (46%), spectinomycin (50%), tylosin-fosfomycin (52%), ciprofloxacin (58%), norfloxacin (67%), oxytetracycline (71%), flumequine (78%), enrofloxacin (82%), neomycin (93%), colistin (94%), pefloxacin (94%), doxycycline (98%) and trimethoprim-sulfamethoxazole (98%) (Osman and Elhariri, 2013). Another observation from Egypt, against isolates of C. *perfringens* from milk and dairy products (Rowayda *et al.*, 2015), displayed that all the isolates were resistant to colistin and ampicillin (100%), followed by lincomycin (91.80%), erythromycin (75.50%), ampicillin-sulbactum (73.40%), neomycin (71.70%), amoxicillin (69.38%), streptomycin (67.34%), spiramycin (63.26%), clindamycin and tetracyclin

(53.06%), cephradine (42.80%), pefloxacin (40.80%), gentamicin (36.73%), norfloxacin (30.60%) and vancomycin (18.36%). Similar, observation was recorded from beef samples in South Africa (Kotsoana and Ateba, 2014) wherein, results revealed that isolates (93.30%-100%) were resistant to penicillin, vancomycin and erythromycin.

The present study were in concurrent with the investigations carried out for antibiotic susceptibility in our country as well as in abroad, while few study in other parts of globe displayed higher resistance to the antimicrobials particularly against the penicillin and cephalosporin group of antibiotics. The resistance to other few antimicrobials like tetracycline, erythromycin, gentamicin, amikacin and floroquinolones is also of major concern. The resistance to the antimicrobials is an alarming sign because these antibiotics are broad spectrum and are commonly used for treatment of the diseases caused by the pathogen. The presence of antibiotic resistance in food borne organisms is directly affecting the human health because these pathogen are not only associated with food poisoning but also many times lead to other systemic infections, which may be fatal in nature. So, the treatment with such kind of antibiotics may not be effective. Besides, it has been observed in many pathogens that antimicrobials resistance increases the virulence/pathogenicity (Beceiro *et al.*, 2013; and Schroeder *et al.*, 2017). The antibiotic resistance is now a global problem and is continuously increasing due to injudicious and indiscriminate use of antimicrobials in therapeutic management and growth promoter. The menace is also of concern because of transfer of R-conjugate plasmid among the bacteria, which promote the resistance in newer antimicrobial (Soge *et al.*, 2009; and Osman and Elhariri, 2013). Therefore, European Union had banned the use of antimicrobials as growth promoters (European Commission, 2005).

Thus, the present study showed occurrence of *C. perfringens* in various meat samples which reflect the poor hygienic condition in retail outlets and slaughter houses. Although, the study didn't reveal any enterotoxigenic strain, but it has been observed by many workers that many non-enterotoxigenic *C. perfringens* are also isolated from gastroenteritis cases. The higher resistance of *C. perfringens* isolates to amikacin, co-trimoxazole, lincomycin and ceftazidime is of concern as these antimicrobials are commonly used in therapeutic management.

CONCLUSION

Overall incidence of *C. perfringens* in meat sample was 23% with buffen-28%, fish meat-24% and chevon and chicken each-20%, in Jabalpur city.

Most of the isolates were sensitive to amoxicillin and ofloxacin, whereas maximum isolates were found to be resistant against amikacin and co-trimoxazole.

The resistance to the antimicrobials is the potential therapeutic challenges in the future, if care is not taken to avoid the selection of multiresistant organisms. It is managed by periodically monitor the susceptibility patterns of *C. perfringens* isolates, considering the possibility that this organism can be a source of resistance genes that can be transferred to other bacterial species, including animal and human pathogens.

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