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Antimicrobial Profile of *Clostridium Perfringens* Isolates from Dairy Products

R.V. Singh* and B. Bist[1]

Department of Veterinary Public Health, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, INDIA
Department of Veterinary Public Health & Epidemiology, CVSc &AH, Jabalpur Madhya Pradesh, INDIA
[1]Department of Veterinary Public Health, CVSc & AH, DUVASU, Mathura, Uttar Pradesh, INDIA

**Corresponding Author: R V Singh; Email: rvsvet24oct@gmail.com.*

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ABSTRACT

The aim of the present study was to observe the antimicrobial profile of isolates of *Cl. perfringens* from pasteurized milk and milk products (icecream and shrikhand) against 16 antibiotics. Antimicrobial profile revealed that few drugs like amikacin, piperacillin, cefuroxime, ceftazidime, cephoxitin, ceftriaxone, chloramphenicol were showing higher sensitivity. Antibiotics like erythromycin and gentamicin were displaying intermediate sensitivity. Drugs like tetracycline, ampicillin, penicillin, co-trimoxazole and cloaxacillin showed higher resistance. While, lincomycin was observed to be almost resistance to *Cl. perfringens* isolates.

Keword: Clostridium, Characterization, Dairy product

Cl. perfringens is a gram positive, sporogenic, encapsulated anaerobic bacteria. The organism is regarded as one of the most widespread and pathogenic bacteria in clostridial family (Songer, 1996). It is ubiquitous in nature and commonly found in the environment and in the gastrointestinal tract of a variety of mammals and birds. It is also recognized as an important pathogen in domestic animals, wildlife and humans. *Cl. perfringens* can cause gas gangrene and food poisoning in humans; necrotic enteritis in poultry; enterotoxemia in lambs and calves; and enteritis in pigs, cattle, dogs, and horses (Radostits *et al.*, 2003, Gurmu *et al.*, 2013).*Cl. perfringens* isolates can be grouped into 5 types (A to E) based on the presence of 4 major toxins- alpha, beta, epsilon and iota (Topley and Wilson, 1998). It also produces enterotoxin, which has been associated with diarrheal disease in some animal species and with food poisoning in humans. The ubiquitous nature of *Cl. perfringens* is responsible for contamination of wide variety of foods like meat,

seafood, vegetables and milk and milk products (Varnam and Evans, 1998). Most of the foods borne infections in humans are associated with meat and meat products but studies have also shown significant presence of pathogen in milk and milk products (Bassiony and Bassiony, 1980). *Cl. perfringens* presence in milk and its product is attributed to environmental contamination viz soil or dust, dung/feces of carrier animal or improper handling during collection, distribution and processing of milk (Jay, 2005). Although, the food poisoning reports are few in number but the organism is associated with spoilage of milk and milk products.

The wide use of antimicrobial agents for growth promotion, prophylactic and therapeutic purpose in animals raised an important question regarding the emergence and spread of antimicrobial resistance primarily in the resident normal enteric flora, including *Cl. perfringens*. Resistance of *Cl. perfringens* isolates has been reported in several countries to bacitracin, tetracycline, clindamycin, lincomycin, and erythromycin. Nevertheless, there is a lack of comprehensive information on *Cl. perfringens* isolates from foods. The purpose of this study was to evaluate the susceptibility of *Cl. perfringens* to different antibiotics.

MATERIALS AND METHODS

Antimicrobial study of *Cl. perfringens* was performed by disc diffusion technique as per procedure described by Bauer *et al.* (1966).

The strains of *Cl. perfringens* were isolated from pasteurized milk, icecream and srikhand. In brief, the isolation of *Cl.perfringens* was done by an agar overlay technique, in which 1ml sample of pasteurized milk or 1ml of diluted sample (25 g sample in 100 ml of distill water viz.1:5) of icecream and srikhand were taken in test tube followed by addition of 5ml of tryptose sulfite cycloserine agar . After solidification of agar, 2ml agar was again added in the test tube followed by overnight incubation at 37^{o}C. The black colonies of 2-3 mm suspected for *Cl. perfringens* were biochemically tested. Biochemically tested isolates of *Cl. perfringens* were inoculated in alternative thioglycollate medium (ATM, Hi media) and incubated overnight (12-15h at 37^{0}C). The inoculated culture was spread on the egg yolk agar (EYagar, Hi media) surface with the help of sterile cotton swab. The antimicrobial-Amikacin, Piperacillin, Cefuroxime, Ceftazidime, Cephoxitin, Ceftriaxone, Cephradine, Chloramphenicol, Erythromycin, Gentamicin, Tetracycline, Penicillin, Ampicillin Co-trimoxazole, Cloaxacillin, Lincomycin (Hi media, Octadisc) were placed on EY agar and incubated under anaerobic conditions for 18-24 h at 37^{0}C. Anaerobiasis in dessicator was created by candle jar method in which, lighted candle was kept inside to absorb the oxygen. The sensitivity/resistance of the cultures was measured by the zone of inhibition around the disc to nearest mm and results were recorded as resistant(R), intermediate (I) and sensitive(S) as per the manufacturer's instructions.

RESULTS AND DISCUSSION

Antimicrobial profile (Table 1) revealed that amikacin, piperacillin, cefuroxime, ceftazidime, cephoxitin, ceftriaxone and chloramphenicol were showing higher sensitivity. Erythromycin and gentamicin were intermediate and tetracycline, penicillin and ampicillin were resistance. Co-trimoxazole and cloaxacillin showed higher resistance. While, lincomycin was observed to be almost resistance to *Cl. perfringens* isolates.

Similar findings have been reported by various workers across the globe (Singh *et al.,* 2005; Tansuphasiri *et al.* 2005; Shojadoost *et al.,* 2010). In a study, Johansson *et al.* (2004) observed 10-76% resistance to tetracycline among *Cl. perfringens* isolates from different European countries. Further, they found resistance against bacteria in those countries where antibiotics are not commonly used. Voidarou (2005) found that isolates of *Cl. perfringens* from infant formula had shown resistance against penicillin, ampicillin, sulfonamides, tetracycline, and gentamicin and cephalosporins. Tansuphasiri *et al.* (2005) observed that *Cl. perfringens* isolates obtained with different sources had displayed resistance to tetracycline (28-78%), ceftriaxone (1-2.5%), chloramphenicol (3%) and penicillin (9%). Similar findings showing multiple drug resistant problems was observed by Shojadoost *et al.* (2010), reported resistance against tetracycline and lincomycin was 80%, gentamicin-53%, erythromycin-30% and ampicillin- 27.5%. In the same study they also revealed that *Cl. perfringens* didn't exhibit resistance against chloramphenicol. Various researchers have observed multi drug resistance problem against the *Cl. perfringens* in several countries. Study by the various workers indicated that resistance problem in tetracycline and/or macrolide is mainly due to transfer of R-plasmid through conjugation among bacteria. The transfer led to development of many resistant genes against the antimicrobial in the same bacteria or different bacteria and thus creating widespread resistance (Holgel *et al*., 2010). Finegold (1989) notified that resistance pattern in anaerobic bacteria may be due to production of beta- lactamase enzyme, inactivating enzyme chloramphenicol acetyltransferase, decrease uptake of drug, plasmid mediated tranferable MDR, changes in the porin molecules in outer membrane, changes in the target organ such as penicillin binding proteins etc. During the present study, it was observed that most of the newer antimicrobials like amikacin, piperacillin, cefuroxime, ceftazidime, cephoxitin, ceftriaxone etc. were displaying higher sensitivity than other antimicrobials and it may be due to lesser use of these drugs in veterinary practice. The multidrug resistance/variation in the sensitive/ resistance pattern of *Cl. perfringens* may be due to excess and injudicial use of antibiotic in prophylactic/therapeutic purpose which leads to overexposure of bacteria to these antimicrobials.

Table 1: Antimicrobial pattern (sensitive/resistance) of isolates of *Cl. perfringens.*

Antibiotics	Antibiotic patterns	Pasteurized milk(5)	Ice-cream (7)	Shrikhand(12)
Penicillin-G (P)	R	3 (60.0%)	5 (71.5%)	5 (41.6%)
	I	2(40.0 %)	1 (14.2%)	5(41.6%)
	S	-	1 (14.2%)	2(16.6%)
Tetracycline (T)	R	3 (60.0%)	4 (57.1%)	5 (41.6%)
	I	-	1 (14.2%)	2 (16.6%)
	S	2 (40.0%)	2 (28.5%)	5 (41.6%)
Co-Trimoxazole (Co)	R	4 (80.0%	6(85.7%)	8 (66.6%)
	I	1 (20.0%)	1 (14.2%)	2 (16.6%)
	S	-	-	2 (16.6%)
Cloxacillin (Cx)	R	3 (60.0%)	5 (71.4%)	9 (75.0%)
	I	1 (20.0%)	1 (14.2%)	1 (8.3%)
	S	1 (20.0%)	1 (14.2%)	2 (16.6%)
Cephradine (Cv)	R	2 (40.0%)	2 (28.5%)	4 (33.3%)
	I	1 (20.0%)	1 (14.2%)	3 (25.0%)
	S	2 (40.0%)	4 (57.1%)	5(41.6%)
Erythromycin (E)	R	2 (40.0%)	2 (28.5%)	5 (41.6%)
	I	-	2 (28.5%)	1 (8.3%)
	S	3 (60.0%	3 (42.8%)	6 (50.0%)
Lincomycin (L)	R	5 (100.0%)	6 (85.7%)	12 (100.0%)
	I	-	1 (14.2%)	-
	S	-	-	-
Cefuroxime (Cu)	R	1(20.0%)	2 (28.5%)	1 (8.3%)
	I	1(20.0%)	2 (28.5%)	2 (16.6%)
	S	3 (60.0%	3 (42.8%)	9 (75.0%)
Amikacin (Ak)	R	-	-	1(8.3%)
	I	-	1 (14.2%)	2(16.6%)
	S	5 (100.0%)	6 (85.7%)	9(75.0%)
Ampicillin (A)	R	3 (60.0%)	3 (42.8%)	8 (66.6%)
	I	1(20.0%)	2 (28.5%)	2(16.6%)
	S	1(20.0%)	2(28.5%)	2(16.6%)
Cephoxitin (Cn)	R	-	4 (57.1%)	-
	I	-	2 (28.5%)	3(25.0%)
	S	5 (100.0%)	1 (14.2%)	9(75.0%)
Ceftazidime (Ca)	R	1 (20.0%)	1 (14.2%)	1(8.33%)
	I	1 (20.0%)	4 (57.1%)	-
	S	3 (60.0%)	2(28.5%)	11(91.6%)
Ceftriaxone (Ci)	R	-	1 (14.1%)	-
	I	2 (40.0%)	2(28.5%)	4(33.3%)
	S	3 (60.0%)	4 (57.1%)	8(66.6%)
Chloramphenicol (C)	R	1 (20.0%)	1 (14.2%)	3(25.0%)
	I	2 (40.0%)	2 (28.5%)	1(8.3%)
	S	2 (40.0%)	4 (57.1%)	8(66.6%)
Gentamicin (G)	R	1 (20.0%)	2(28.5%)	4(33.3%)
	I	2 (40.0%)	2(28.5%)	3(25.0%)
	S	2 (40.0%)	3 (42.8%)	5(41.6%)
Piperacillin (Pc)	R	1 (20.0%)	1 (28.5%)	2(16.6%)
	I	1(20.0%)	1(14.2%)	4(33.3%)
	S	3 (60.0%)	5 (71.4%)	5(40.1%)

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