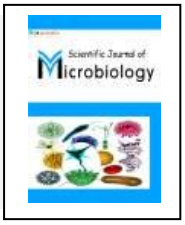
Sjournals
Scientific Journals
Scientific Journal of Microbiology (2013) 2(4) 83-88
ISSN 2322-2948

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com



Original article

Isolation, identification and characterization of *Clostridium perfringens* from lamb dysentery in Dinajpur district of Bangladesh

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ARTICLE INFO

Article history:
Received 03 April 2013
Accepted 18 April 2013
Available online 29 April 2013

Keywords:
Clostridium perfringens
Lamb
Isolation
Identification
Antibiogram

ABSTRACT

The present study was conducted for isolation, identification and characterization of *Clostridium perfringens* organism from lamb dysentery in Dinajpur district of Bangladesh. Affected sheep showed clinical signs as dysentery, diarrhea, anorexia, weakness, and dehydration. A total of 20 faeces and rectal swab were collected from different sheep farms of different area of Dinajpur District of Bangladesh. Faeces and rectal samples were collected aseptically and microbial examination was done by using Gram's staining, cultural and biochemical techniques in the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. Out of 20 faeces and rectal swab samples 5 were found to be positive. The percentages of positive sample were 25%, 40%, 12.5% and 33.33% respectively in Rezanur sheep farm, Khalilur sheep farm, Hasan sheep farm and Mojammal sheep farm. Antimicrobial susceptibility test was performed by discs diffusion method. Most of the isolated *Clostridium perfringens* were susceptible to ciprofloxacin, levofloxacin and penicillin. However,

most of the isolated *Clostridium perfringens* were resistant to gentamycin, streptomycin and chloramphenicol. The antimicrobial susceptibility testing reaveled that penicillin, ciprofloxacin and levofloxacin were most efficacious followed by amoxycillin and azithromycin. So, it may be recommended that penicillin, levofloxacin and ciprofloxacin in optimum doses would resolve most cases of lamb dysentery in the Dinajpur district of Bangladesh.

1. Introduction

Clostridium perfringens is a Gram-positive anaerobic bacterium and able to form spores. It is widespread in the environment, commonly found in the intestines of animals and humans and can be pathogenic. In humans, it can cause gangrene and gastrointestinal diseases (for instance food poisoning and necrotic enteritis), whereas in other animals, gastrointestinal and enterotoxemic diseases occur more frequently (Petit et al., 1999; Gkiourtzidis et al., 2001).

Clostridium perfringens produces disease in sheep and goats, most of which are generically called enterotoxemia. This micro-organism is classified into five types (A, B, C, D and E) according to the production of four major toxins, namely alpha, beta, epsilon and iota. Two other major toxins (i.e. enterotoxin and beta 2) can also be produced by all types of *Clostridium perfringens*, although they are not used for the typing of this microorganism. *Clostridium perfringens* can be a normal inhabitant of most animal species (Niilo, 1980) including humans the but when the intestine is altered by sudden changes in diet or other factors. *Clostridium perfringens* proliferates in large numbers and produces several potent toxins. These toxins can act locally (i.e. beta toxin producing necrotic enteritis in lambs); can be absorbed into the general circulation producing systemic effects (i.e. epsilon toxin producing cerebral microangiopathy in lambs); or can act both locally and systemically (i.e. epsilon toxin producing diphtheritic colitis and microangiopathy in unvaccinated goat kids) usually with devastating effects on the host (Uzal, 1996; Lewis, 2000).

Clostridium perfringens Type B lamb dysentery caused by *Clostridium perfringens* Type B is infrequently diagnosed. The disease is seen in lambs, usually less than 2 weeks of age, and is characterised by a haemorrhagic enteritis. Lambs are usually found dead. Affected lambs show signs of abdominal pain and have fluid, bloodstained faeces. The diseases can be controlled by maintaining high level of biosecurity, routine different types of cultural and biochemical test and screening of lambs positive to *clostridium perfringens*. Therefore, the present study was undertaken with a view to isolate, identify and characterize *Clostridium perfringens* organism from lamb dysentery in Dinajpur district of Bangladesh.

2. Materials and methods

2.1. Selection of study area

The research work was carried out in Rezanur's sheep Farm in Matasagar, Kholilur's sheep farm in Pulhat, Hasan's sheep farm in Gopalgang, Mojammel's sheep farm in Pulhat, Dinajpur during the period from July to December 2012.

2.2. Isolation and identification of *Clostridium perfringens* from affected lamb

2.2.1. Collection of field sample

A total number of 20 field samples comprising faeces and rectal swab of affected lamb of study areas were aseptically collected and carried to the Microbiology laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology Universiy, Dinajpur. Isolation and identification of *Clostridium perfringens* were performed as per procedures described by Marchant and Packer (1967), OIE (2000) and Calnek (1997).

2.2.2. Media for culture

All the samples were primarily cultured in Nutrient broth (NB) then subcultured in the Blood agar (Hi- Media, India) and Cooked Meat Media as per procedures described by Marchant and Packer (1967), OIE (2000) and Calnek (1997).

2.2.3. Morphological study

The presumptive colonies of *Clostridium perfringens* in different media were characterized microscopically by using Gram's staining (Marchant and Packer, 1967).

2.2.4. Biochemical characterization

Several biochemical tests such as carbohydrate fermentation test, triple sugar iron (TSI) agar slant reaction, methyl – red and voges –proskauer (MR-VP) test and indole reaction as per procedures described by Marchant and Packer (1967), OIE (2000) and Calnek (1997).

2.2.5. Antibiogram study

C. perfringens isolates presenting *in vitro* susceptibility to 10 different antimicrobial agents were tested by a disc diffusion method (Wust, 1977). The antimicrobial agents used are ciprofloxacin, amoxycillin, streptomycin, penicillin, chloramphenicol, enrofloxacin, gentamicin, levofloxacin, azithromycin and neomycin.

3. Results

The results of isolation and identification of *C. perfringens* from suspected lamb by using staining, cultural and biochemical tests are summarized in Tables 1, 2, 3 and 4. A total of 20 faeces sample were culture on different bacteriological media. The percentage of positive case in different farms were 25%, 40%, 12.5% and 33.33%, respectively. A total of 5 isolates were confirmed as *C. perfringens* by Gram's staining, cultural properties and biochemical tests in this study. The isolated *C. perfringens* produced hemolytic colony on blood agar and blackened colony on cooked meat media. Grams staining from blood agar revealed that Gram-positive, violet colored, small rod shaped appearance, arranged in single or paired under the microscope. The non-motility of *C. perfringens* was confirmed by hanging drop method. The isolated *C. perfringens* fermented sucrose, maltose, lactose and glucose. The isolated *C. perfringens* were negative to indole and MR-VP test. However, the isolated *C. perfringens* showed positive reactions in TSI agar slant. In Triple Sugar Iron (TSI) slant agar, the slant was turned yellow due to the increased level of acid production indicating carbohydrate fermentation. H_2S was also produced due to the reaction of sulphur containing compounds. Hydrogen sulphide reacted with the ferrous sulphate of the medium producing ferric sulphide giving a black precipitate to the upper layer of the slant.

The results of antimicrobial susceptibility of the isolated *C. perfringens* are summarized in Table 5. Out of 5 *C. perfringens,* 5 isolates were susceptible to ciprofloxacin, penicillin and levofloxacin. On the other hand, out of 5 *C. perfringens,* 100% isolates were resistant to streptomycin, chloramphenicol and gentamicin. Furthermore, 40% isolates were susceptible to amoxycillin, enrofloxacin, azithromycin and neomycin. Moreover, 60% isolate were resistant to amoxycillin, enrofloxacin, azithromycin and neomycin.

Table 1
Isolation and identification of *Clostridum perfringens* from suspected lamb by using staining, cultural and biochemical tests.

Study area	Study population	Sources of samples	No. of sample tested	No. of positive isolates	% of positive case
Rezanur's sheep farm	20	Faeces and rectal swab	4	1	25%
Khalilur's sheep farm	25	Faeces and rectal swab	5	2	40%
Hasan's sheep farm	30	Faeces and rectal swab	8	1	12.5%
Mojammal's sheep farm	25	Faeces and rectal swab	3	1	33.33%

Table 2
Cultural characteristics of *Clostridium perfringens* isolated from affected lamb in different culture media.

Media used	Colony characteristics	Isolated organism
Nutrient agar	Translucent, opaque, smooth colonies	*Clostridium perfringens*
Nutrient broth	Turbidity in the broth	
Blood agar	Hemolytic colony	
Cooked meat media	Blackened colour	

Table 3
Characteristics of *Clostridium* isolates by Gram's staining method and motility test.

Isolate	Motility	Morphology	Staining
Clostridium perfringens	Non motile	Small rod, single or paired, violet in color	Gram-positive

Table 4
Results of biochemical tests.

Different biochemical tests	Result
Fermentation reaction with sucrose	+
Maltose	+
Lactose	+
Glucose	+
Mannitol	-
Indole	-
MR	-
TSI	+
VP	-
H_2S	+
Dulcitol	-

Legends: MR= Methyl Red; VP= Voges-Proskaure; TSI = Triple Sugar Iron; + = Positive; - =Negative.

Table 5
Results of antimicrobial susceptibility test of the isolated bacteria (n = 05).

Antimicrobial agent	No (%) of *C. perfringens*		
	Susceptible	Intermediate	Resistant
Ciprofloxacin	5 (100)	0 (0)	0 (0)
Amoxycillin	2 (40)	0 (0)	3 (60)
Streptomycin	0 (0)	0 (0)	5 (100)
Penicillin	5 (100)	0 (0)	0 (0)
Chloramphenicol	0 (0)	0 (0)	5 (100)
Enrofloxacin	2 (40)	0 (0)	3 (60)
Gentamicin	0 (0)	0 (0)	5 (100)
Levofloxacin	5 (100)	0 (0)	0 (0)
Azithromycin	2 (40)	0 (0)	3 (60)
Neomycin	2 (40)	0 (0)	3 (60)

4. Discussion

Clostridium perfringens is a widely distributed pathogen known to cause many human and animal diseases. Domestic animals are known to be sources of human food poisoning; to decrease or eliminate this risk, strategies must be developed to prevent infected animals from entering the food chain (Johnson, 1997; Mcdonel, 1986). The study was conducted for isolation, identification and antibiogram study of *Clostridium perfringens* from lambs dysentery. The research work was carried out from Rezanur sheep farm in Matasagar, Khalilur sheep farm in Pulhat, Hasan sheep farm in Gopalgang and Mojammel sheep farm in Pulhat of Dinajpur District and the samples were brought to the Microbiology laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University. Dinajpur, Bangladesh, during the period of July to December 2012. During this study the isolate produced translucent, opaque, smooth colonies on nutrient agar. On nutrient broth produced turbidity, on blood agar produced hemolytic colony and blackened colony on cooked meat media. These results are in agreement with the report of Cheung et al. (2004) and Tillotson et al. (2002).

Grams staining from blood agar revealed that Gram-positive, violet colored, small rod shaped appearance, arranged in single or paired under the microscope. Isolate was found to be non motile when examined under microscope. The characteristics of lamb *Clostridium* isolates by Gram's staining method and motility test are presented. The isolated *C. perfringens* fermented sucrose, maltose, lactose and glucose. The isolated *C. perfringens* were negative to indole and MR-VP test. In the MR test the absence of the red colour in the media after the addition of 3 ml methyl red with the cultural growth was observed and thus indicating the isolated *Clostridium* were negative for MR. In the Voges-Proskauer (V-P) test, no change of colour of the media was observed after the addition of 3 ml of 3% potassium hydroxide to 3 ml V-P broth media with the cultural growth of the isolated *Clostridium perfringenes* and thus indicated that the isolated *Clostridium* from lamb was negative for V-P test. These results are similar with the respects of Galizzi et al. (2001) and Gibert et al. (2001).

Out of 5 *C. perfringens,* 5 isolates were susceptible to ciprofloxacin, penicillin and levofloxacin. On the other hand, out of 5 *C. perfringens,* 100% isolates were resistant to streptomycin, chloramphenicol and gentamicin. Furthermore, 40% isolates were susceptible to amoxycillin, enrofloxacin, azithromycin and neomycin. Moreover, 60% isolate were resistant to amoxycillin, enrofloxacin, azithromycin and neomycin. These results are partially similar to the findings of Singh et al. (2010) and Schotte (2004).

5. Conclusion

It may be recommended from this study that ciprofloxacin and penicillin in optimum doses would resolve most cases of lamb dysentery in the Dinajpur district of Bangladesh. Lamb dysentery in a sheep farm sends alarm to a farmer and treatment is given immediately to control it. The incidence of lamb dysentery should be prevented by providing good husbandry practice with bio-security measure. Check list for lamb dysentery should be prepared and accordingly proper treatment should be recommended.

Acknowledgement

The authors wish to express their heartfelt thanks to Professor Dr. M. Mostafizer Rahman, Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur – 5200, Bangladesh for critical reading of this manuscript.

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