IN VITRO LECITHINASE ACTIVITY AND ANTIBIОGRAM OF CLOSTRIDIUM PERFRINGENS ISOLATED FROM BROILER CHICKENS

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ABSTRACT

Clostridium perfringens is well known as the etiological agent of necrotic enteritis in chickens. C. perfringens were detected in intestinal contents from forty nine cases of enteritis in broiler chicken. In the present study in-vitro lecithinase activity of 49 isolates and sensitivity of 30 isolates to 11 antimicrobial agents were assessed. A total of forty four (89.79%) isolates expressed lecithinase activity. Antibiogram studies showed highest sensitivity to fluoroquinolones like ciprofloxacin (93.3%), followed by enrofloxacin (86.6%) and moderate sensitivity to chloramphenicol (76.6%), oxytetracycline (70%) and co-trimoxazol (63.3%). The organisms showed 100% resistance to streptomycin and neomycin.

Key words: Clostridium perfringens, lecithinase activity, antibiogram

MATERIALS AND METHODS

Isolation of C. perfringens: Reference strains of C. perfringens type ‘A’ and type ‘C’ were procured from the Division of Biological Standardization, Indian Veterinary Research Institute (IVRI), Izatnagar. The strains were maintained as stock culture in thioglycollate broth and were periodically tested for purity, morphology and biochemical characteristics. Anaerobic jars were used for anaerobic incubation wherever required. Burning candle was used in anaerobic jar and it was tightly sealed with parafilm to create anaerobic environment. The intestinal contents collected from 4-5 enteritis affected birds in a flock were pooled to make one pooled sample (pooled sample will be referred to as sample in the manuscript). Sixty eight pooled samples of intestinal contents were processed for isolation of C. perfringens using one step enrichment and selective plating (Varnam and Evans 1991). Ten gram of faecal sample was inoculated in 90 ml of thioglycollate enrichment medium (Hi media) in sterile flask. After mixing for 2-3 min, homogenized sample was heated at 75°C for 20 min, followed by incubation at 37°C for 24 h. For isolation of C. perfringens, an agar overlay technique in sterile test tubes was used using Tryptose Sulphite Cycloserine agar [TSC] (Varnam

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Thioglycollate enriched inoculum (0.1ml) was taken in a sterile straight test tube and 5 ml of sterile TSC agar was added with thorough mixing. It was allowed to solidify for 5-10 min and again 2-3 ml of TSC agar was overlaid. The test tube was then incubated at 44-45°C for 18-24 h. The black cotton wool like colonies of 2-3 mm in size suspected for *C. perfringens* were taken out with the help of a loop preferably from subsurface and incubated at 37°C in thioglycollate broth for 24 h and the organisms were subjected to biochemical tests for confirmation. Suspected isolates of *C. perfringens* were identified on the basis of grams staining, hanging drop motility test, gelatin liquefaction and nitrate reduction as per standard procedure recommended by Cowan and Steel (1974). The isolates were also subjected to lecithinase activity on egg yolk agar and for haemolysis on blood agar.

**Lecithinase Activity (Nagler Test):** Lecithinase production was detected on egg yolk agar plate by spot inoculation according to method of Willis and Hobbs (1958). Overnight grown culture of *C. perfringens* in thioglycollate broth was inoculated on egg yolk agar plate. It was followed by 24 h incubation under anaerobic condition at 37°C. Plates were observed for zone of opalescence around colonies indicating positive result.

**Antibiogram assay:** In-vitro antibiogram study was carried out on 30 isolates of *C. perfringens*. The assay was performed by disc diffusion technique as per procedure described by Bauer et al. (1966). The antibiotics used in this study include ciprofloxacin, enrofloxacin, chloramphenicol, oxytetracycline, cotrimoxazole, cefoxime, gentamicin, ampicillin, colistin, streptomycin and neomycin. The overnight grown culture of *C. perfringens* in thioglycollate broth were spread on the surface of Mueller Hinton agar surface with the help of sterile cotton swab and after drying, antibiotic discs were placed and the plates were then incubated under anaerobic condition for 24 h at 37°C. The sensitivity/resistance of the cultures was measured by measuring the zone of inhibition around the disc and results were analysed as sensitive or resistant according to manufacturer’s (Hi-Media) instructions.

**RESULTS AND DISCUSSION**

A total of 68 samples of intestinal contents from the enteritis affected broiler birds brought to D.I.Lab, Hisar were screened for isolation of *C. perfringens*. Black wool like colonies of 2-3 mm in size was obtained in TSC agar tubes after enrichment in thioglycollate broth. The organisms in these colonies were confirmed as Gram positive, non-motile, rod shaped bacteria with square ends. The *C. perfringens* isolates were further characterized by biochemical tests. All the isolates were found to be positive for gelatin liquefaction and nitrate reduction. In hemolytic positive cases, hemolysis was seen around the colonies and 41 isolates (83.67%) showed the expression of haemolysin. Haemolysin and lecithinase negative variants of *C. perfringens* isolates were observed in our study. The results of isolation revealed an overall positivity of 72.05% as isolation could be made from 49 samples. Previous studies have reported varied incidence of *C. perfringens* in poultry as 82 to 84% as reported by Miwa et al. (1996). Shane et al. (1984) and Craven et al. (2001a,b) reported that when intestinal contents of broiler chickens were analyzed for presence of *C. perfringens*, approximately 75% to 95% of broiler chickens were found positive. In present investigation, enrichment with heat treatment at 75°C for 20 min followed by use of selective TSC agar was employed for the isolation of *C. perfringens*. The heat treatment used in this protocol, killed most of the heat sensitive pathogenic and non-pathogenic organisms thereby allowing the growth of *C. perfringens*. The use of enrichment before selective plating has been reported to show better recovery percentage of *C. perfringens*. (Fujisawa et al. 2001).

All the 49 isolates of *C. perfringens* when tested for lecithinase activity on egg yolk agar, 44 isolates (89.79%) showed a zone of opalescence around colonies (Fig 1) exhibiting the lecithinase activity in them and five isolates were negative for the same. Out of 49 isolates, 39 were positive both for lecithinase and hemolysin, 5 positive for lecithinase but negative for hemolysin, 2 negative for lecithinase but positive for hemolysin and 3 were negative for both. These observations are in agreement to previous reports where some *C. perfringens* strains were also found...
to be lecithinase negative and non-haemolytic (Skjelkvale, 1979). The biochemical variants of *C. perfringens* have also been reported by Ampratwun (1993) and Skjelvale et al. (1979).

Thirty (30) isolates of *C. perfringens* out of 49 obtained during study were subjected to in-vitro antibiotic sensitivity against eleven commonly used antibiotics. The results of antibiogram assay revealed that *C. perfringens* showed highest sensitivity to fluoroquinolones viz. ciprofloxacin (93.3%) and enrofloxacin (86.6%) (Fig 2). The other antibiotics that were found to be sensitive include chloramphenicol (76.6%), oxytetracycline (70%), co-trimoxazol (63.3%) cefatoxime (40.0%), gentamicin (36.6%), ampicillin (33.3%) and colistin (13.3%). All the isolates were highly resistant to streptomycin and neomycin (100%).

Emergence of resistant strains against antimicrobial agents has become a public health concern all over the world (Witte, 1998). Constant and indiscriminate use of antibiotics in both veterinary and human medicine, especially in developing countries coupled with current knowledge of transfer of antibiotic resistance between various bacteria makes it essential to monitor the susceptibilities of known pathogenic bacteria to antibiotics (Linton, 1977). This study of antibiogram with eleven commonly used antibiotics showed that *C. perfringens* isolates were sensitive to ciprofloxacin, enrofloxacin, chloramphenicol, oxytetracycline, and Co-trimoxazol whereas moderately resistant for cefatoxime, gentamycin, ampicillin, colistin and completely resistant to streptomycin and neomycin.

**REFERENCES**


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