ABSTRACT

Clostridium perfringens is a gram-positive, anaerobic spore-forming bacteria which is present ubiquitously in the environment. By virtue of different toxins produced by C. perfringens, it can cause different gastrointestinal disorders in human, animals, and birds which might lead to mortality. In the present epidemiological study, a total of 160 isolates have been recorded at a rate of 24.92% with type A (134), type B (1), type C (17), type D (5) and type E (3) isolates. Other toxin genes viz. cpb2, cpe, and netB are present in 48%, 1.8%, 3.1% of total isolates respectively. Isolation rates from different host were found to be Human (33.8%), Cattle (20.8%), Goat (18.3%), Pig (32.7%), Poultry (9.3%), Dog (34.6%) and Captive wild animals (37.5%). Isolation rate from the different North Eastern States of India was found to be Arunachal Pradesh (22.2%), Assam (22.8%), Meghalaya (29.7%), Manipur (42.8%), Nagaland (30.3%) and Mizoram (0%). mPCR developed for simultaneous detection of seven toxin genes (cpa, cpb, cpb2, etx, iap, cpe, and netB) have been found to be more sensitive over the traditional six gene mPCR with detection up to 12.5ng of genomic DNA. Molecular epidemiology study of all 160 isolates was conducted by rep-PCR using the Diversilab® system. Clonality of the isolates was determined based on the presence or absence of bands as well as the intensity of bands by using Pearson Correlation (PC) or Kullback-Liebler (KL) method. KL method of similarity analysis was found be better over PC method. However, the PC method appears to be more efficient among isolates belonging to the same toxin type. The study was further extended to analyze the expression levels of etx gene in a different medium. The highest expression of etx has been observed in laboratory-made ε–exp-1 media. The epsilon toxin (ETX) produced was found to have an LD50 value of 330 ng/kg body weight. Epsilon toxoid (εox) was prepared by chemical treatment and used for the preparation of calcium phosphate nanoparticle (CaP-εox NP) with 68.6 nm in diameter and -23mV of surface potential. Nanoparticles could be loaded with εox up to 20.71 µg/mg. CaP-εox NPs as vaccine composition is found to be better over traditional aluminum hydroxide adjuvant based and commercial Raksha-ET® vaccine. The 14th-day antibody titer of 4.90±0.002 (log mean titer ± SE) in CaP-Etx group with CaP-εox NPs could protect up to 500X LD50 dose of ETX.