

ORL- D&H-06**Diversity of *Clostridium* Bacteria from Commercially Important Ichthyofauna Inhabiting at Eutrophic Lake of Kashmir Himalaya**

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The current study documented the presence of *C. perfringens* in the aquatic environment of the Dal Lake in the Kashmir valley to estimate the presence of bacteria and its load. Using species-specific 16S rRNA gene amplification, all the water samples from 3 basins of Dal lake were found to be positive for *C. perfringens* confirming the presence of *C. perfringens* in the lake. The toxinotyping of *C. perfringens* isolates carried out by multiplex-PCR targeting six toxin genes (*cpa*, *cpb*, *etx*, *itx*, *cpb2* and *cpe*) revealed the presence of *cpa* gene and thus were designated as toxinotype A. The phylogenetic analysis of the protein sequences of *cpa* toxin revealed a close relationship with corresponding AA sequences of *C. perfringens* strains reported from India, Egypt and China. The overall *C. perfringens* load in water samples was found to be in the range of 9.2×10 CFU/ml to 1.87×10^2 CFU/ml, which is much less than the critical CFU level of *C. perfringens* (10^5 - 10^8). The highest average CFU/ml value was found at Dalgate (1.87×10^2), followed by Telbal Nallah (1.78×10^2), Dhobi Ghat (1.20×10^2), Centaur Lake View (1.05×10^2) and Char-Chinari (9.2×10), respectively. Antibiotic sensitivity test of *C. perfringens* isolates revealed high resistance to more than 3 antimicrobials with amoxicillin/clavulanic acid, chloramphenicol and ciprofloxacin as being most sensitive. The myriad ways in which people use the lake along with the numerous pollutant-generating activities have stressed the lake ecosystem in diverse ways. Water of the lake is continuously being fed with human excreta through sewage dumping, although some partial treatment of the sewage is done via treatment plants. The lake is in a serious state of eutrophication mostly cultural and ways and means for lake restoration have not proved to be fruit full till date.

Toxinotyping of *C. perfringens* isolates from water samples by multiplex-PCR revealed the presence of type A strain (carrying only α -toxin gene). Thus water accounts to be reflected as an imminent source of *C. perfringens* type A infection to human populaces via the food chain and might pose a serious public health concern as the lake is used as a biggest vegetable bowl of city. Phylogenetic analysis of the *cpa* toxin genes of *C. perfringens* revealed 95% to 98% homology with corresponding GenBank published sequences and close relationship with corresponding AA sequences of *C. perfringens* strains reported from India, Egypt and China. Avg Bacterial Load= 9.2×10 CFU/ml to 1.87×10^2 CFU/ml, much less than critical CFU level (10^5 - 10^8 CFU/ml). drinking, cooking or sanitary purposes. The antibiogram pattern of the *C. perfringens* type A isolates revealed a higher antimicrobial resistance to amikacin, ceftriaxone, metronidazole, norfloxacin and tetracycline.

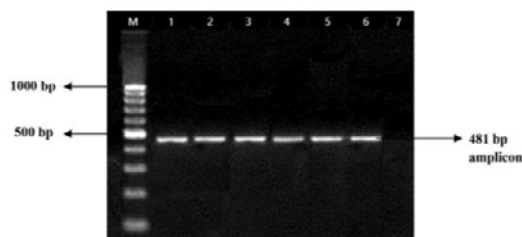


Figure 1. Detection of 16s rRNA



Figure 2. In vitro antibiotic sensitivity pattern