Abstract

Abstract

Diarrheal diseases caused by *Vibrio* spp. and *Shigella* spp. are the common cause of death in developing countries. Increase in drug resistance in these pathogens hampers the treatment and therefore, it is important to understand the mechanisms of drug resistance in these isolates. Role of mobile genetic elements in imparting multiple drug resistance was investigated in clinical isolates of *Vibrio fluvialis* and *Shigella* spp. Thirteen isolates of *V. fluvialis* (2002 and 2006) and ninety-five clinical isolates of *Shigella* spp (2001 to 2010) were obtained from the Infectious Diseases Hospital, Kolkata, India. All the isolates displayed drug resistance with varying antibiograms. However, resistance to nalidixic acid, trimethoprim, streptomycin and co-trimoxazole were most common in *Shigella* population while ampicillin and neomycin resistance was common in *V. fluvialis* isolates. The role of mobile genetic elements as well as chromosome-borne resistance factors was analysed in detail.

Integron analysis in *V. fluvialis* isolates indicated that only one *V. fluvialis* (BD146 of 2002) carried two typical class 1 integrons. One class 1 integron residing on a low copy number plasmid carried *arr*3-*cml*A-bla_{OXA10}-*aad*A1 gene cassette responsible for resistance to rifampicin, chloramphenicol, beta-lactam and aminoglycoside respectively. Another class 1 integron carried a putative exporter protein. Apart from low copy number plasmid, *V. fluvialis* BD146 also carried a high copy number plasmid pBD146 of 7.5 kb. Sequence analysis of pBD146 revealed the presence of genes encoding an integrase (BDint), toxin-antitoxin (*parE/parD*), a replicase, trimethoprim resistance (*dfrVI*) and quinolone resistance (*qnrVC5*). pBD146 showed 99% sequence similarity with pVN84 from *V. cholerae* O1 of Vietnam, 2004 and a plasmid from *V. parahaemolyticus* v110 of Hong Kong, 2010.

Conjugation and transformation experiments in four *V. fluvialis* isolates proved the ability of their resident plasmids to get transferred to another host imparting their antibiotic resistance traits to the transconjugants and transformants. Efflux pumps were minimally involved in the drug resistance phenotype for ampicillin, chloramphenicol, kanamycin and tetracycline. The extended spectrum beta lactamases (ESBLs) activity were observed in representative isolates (BD146 and L15318).

Mutation in gyrase A (S_{83}→ I) and ParC (S_{83}→L), *qnrVC5* (in BD146, L10734 and L9978) and *aac(6')-Ib-cr* (in BD146) genes were found to contribute towards quinolone resistance.

In *Shigella* isolates, preponderance of class 2 and atypical class 1 integrons was observed. Typical class 1 integron was present in only one *S. sonnei* isolate and harbored trimethoprim resistance-encoding gene *dfrV* while atypical class 1 integrons harboured *dfrA1–aadA* or bla_{OXA}-*aadA* gene cassettes responsible for resistance to trimethoprim, aminoglycosides and β-lactams. Class 2 integrons harboured either *dfrA1-sat-aadA* or *dfrA1-sat* gene cassettes. Most importantly, a novel gene cassette array *InsE-InsO-dfrA1-sat* was found in class 2 integron of *S. sonnei* NK4846. Many of the resistance traits for antibiotics such as trimethoprim, co-trimoxazole, kanamycin, ampicillin and tetracycline were transferred from parent *Shigella* isolates to recipient *E. coli* during conjugation establishing the role of plasmids in horizontal transfer of resistance genes. Multiple mutations such as S_{80}→I, S_{83}→L, D_{87}→G/N/Y in quinolone resistance determining regions of topoisomerases from the representative quinolone resistant isolates could explain the spectrum of MIC values for various quinolones.

Therefore, the present study has indicated that the mobile (plasmids, integrons and quinolone resistance genes named *qnr*) as well as innate genetic elements (mutations in topoisomerases) played an important role in dissemination of drug resistance in clinical isolates of *Shigella* spp. and *Vibrio fluvialis*. The study has clearly established that these diarrhea-causing pathogens are replete with multiple drug resistance genes/mechanisms and therefore points out the requirement for prudent use of antibiotics.