Summary and Conclusions

Vibrio spp. and *Shigella* spp. are pathogens that cause diarrhoea or dysentery and they are the common cause of death in developing countries and infant deaths worldwide. For shigellosis, antibiotics are the mainstay for the treatment while in cholera treatment is oral rehydration therapy and administration of antibiotics. The increasing occurrence of resistant bacteria gradually renders antibiotics ineffective in treating infections and has enormous health and economic implications worldwide. Resistance to antibiotics in microbes has been attributed to genetic factors that could be inherent for the bacteria or acquired by them due to mobile genetic elements (MGEs). MGEs include plasmids, integrons and integrating conjugative elements (ICEs) which are potent vectors for acquisition and dissemination of antibiotic resistance genes among the bacterial populations. Bacteria are inherently resistant to an antibiotic through multiple mechanisms such as mutations in target genes, efflux pump activity and mutations in porins. The present study was undertaken to understand the extent of multiple drug resistance (MDR) in *Shigella spp.* and *Vibrio fluvialis,* and unravel the genetic factors responsible for emergence and dissemination of MDR. Various genetic factors borne on chromosomes or plasmids or integrons were deciphered as reasons for the observed MDR phenotypes.

The work was carried out with 13 clinical isolates of *V. fluvialis* and 95 clinical isolates of *Shigella spp*. which were procured from National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India. These clinical isolates were obtained from the patients with acute diarrhoea or bloody diarrhoea, admitted to the Infectious Diseases Hospital, Kolkata, India. This study was carried out in two parts as described below:

Role of mobile genetic elements in multidrug resistant *V. fluvialis* isolates

Out of thirteen *V. fluvialis* isolates, twelve were from the year 2006 and one from 2002. These isolates were analysed for antibiogram, mobile genetic elements-borne and chromosome- borne resistance mechanisms.

Antibiotic susceptibility test of *V. fluvialis* BD146 (2002) showed complete resistance to twelve drugs and intermediate susceptibility to the two drugs. The antibiotic screening, conjugation, transformation and plasmid analysis indicated the

presence of two types of plasmids: a low copy number plasmid and a high copy number plasmid of 7.5 kb. Integrons (class 1 to class 4) and SXT element as carrier of drug resistance genes were analysed by PCR. Results revealed that only two class 1 integrons were detected in *V. fluvialis* BD146. One integron carried the putative exporter protein, while the other integron resident on low copy number plasmid carried various gene cassettes responsible for rifampicin, ampicillin, chloramphenicol, gentamicin and kanamycin resistance. The high copy number plasmid pBD146 of *V. fluvialis* BD146 carried genes like *dfrVI* (responsible trimethoprim resistance), *qnrVC* (responsible quinolone resistance), *parE/parD* (toxin-antitoxin system), a replicase and an integrase *BDint*. Quinolone resistace was found associated with the mutations in topoisomerase and plasmid mediated quinolone resistance (*qnrVC5* and *aac(6')-Ib-cr*) genes. Efflux pumps contributing to drug resistance phenotype was analysed by synergy test and results revealed that these were minimally active for drug resistance. The extended spectrum beta lactamase (ESBL) and AmpC beta lactamase activity was observed in this isolate by MIC analysis.

In another study, antibiotic susceptibility tests of 12 *V. fluvialis* isolates (2006) revealed that all the isolates displayed drug resistance with varying antibiograms. Resistance to ampicillin and neomycin was common to all of them. Pulsed Field Gel Electrophoresis (PFGE) analysis of three isolates revealed similar band pattern implicating that there could be derived from the same clone. MGEs such as class 1 to class 4 integron and SXT element were found to be absent in these isolates. The drug-resistance traits for ampicillin, co-trimoxazole, ciprofloxacin, chloramphenicol, neomycin, trimethoprim, streptomycin, etc were transferable by conjugation, indicating the role of plasmids in horizontal transfer of drug resistance genes. Mutations in topoisomerase gyraseA (S_{83}→I), ParC (S_{85}→L) and the presence of *qnrVC* gene were found to contribute towards quinolone resistance. Efflux pump activity by synergy tests revealed that these were operative for the resistance phenotype of ampicillin and tetracycline. The ESBL activity was observed in L15318 isolate by MIC analysis.

Therefore, the present study clearly showed that plasmids and class 1 integrons along with efflux pump and topoisomerase mutations played an important role in drug resistance phenotypes in the clinical isolates of *V.fluvialis*.

Role of various mobile genetic elements in multidrug resistant *Shigella* isolates

Ninety five clinical isolates of *Shigella* were analysed to understand the frequency and patterns of antimicrobial resistance. In this population, *S. flexneri* and *S. sonnei* were found to be more prevalent as compared to the other two species *S. dysenteriae* and *S. boydii*. Majority of these isolates were found resistant to trimethoprim, nalidixic acid, streptomycin, kanamycin, co-trimoxazole, tetracycline, ciprofloxacin and norfloxacin. PFGE analysis of these *Shigella* isolates revealed that *S. sonnei* had more clonally related isolates as compared to the other *Shigella* isolates. These isolates were further analysed for the presence of mobile genetic elements imparting drug resistance by PCR, sequencing and conjugation.

In the present study, typical class 1 integron was present only in one *S. sonnei* isolate and harboured the dihydrofolate reductase gene (*dfrV*) responsible for trimethoprim resistance. Forty one isolates comprising of *S. flexneri, S. dysenteriae* and *S. boydii* harboured the atypical class 1 integron and harbored the genes responsible for beta-lactam resistance (bla_{OXA}), trimethoprim resistance (*dfrA*) and aminoglycoside resistance (*aadA*). Class 2 integron was prevalent in *Shigella* isolates as it was present in 83% of all these isolates. Class 2 integron carried gene cassettes for trimethoprim resistance (*dfrA*), streptothricin resistance (*sat*) and aminoglycoside resistance (*aadA*) except *S. sonnei* NK4846 which harbored a novel cassette array *InsE-InsO-dfrA1-sat* (InsE-InsO: transposase) on a class 2 integron. The class 1 and class 2 integrons-borne resistance traits (trimethoprim, kanamycin and ampicillin), along with other resistance traits (cotrimoxazole, tetracycline, streptomycin etc.) were transferable during conjugation, establishing the role of plasmids in horizontal gene transfer.

Apart from MGE-borne antibiotic resistance, chromosomal mutations in topoisomerase (S_{80}→I, S_{83}→L, D_{87}→G/N/Y) in quinolone resistance determining regions defined quinolone resistance in some of the isolates. However, synergy test reveals no efflux pump activities in these isolates.

Overall, plasmids, class 1 and class 2 integrons, and mutation in topoisomerases seemed to play an important role in drug resistance of these *Shigella* isolates.

The study pursued here has indicated the prevalence of highly and multiply drug resistant pathogens belonging to the genus *Vibrio* and *Shigella* from the region of

Kolkata. Detailed genetic studies has indicated interplay of a large number of genetic factors such as plasmids, integrons and mutations in topoisomerases being responsible for the prevalence of high drug resistance in these pathogens.