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SUMMARY AND CONCLUSION

This study involved the morphological, biochemical and molecular characterization of the Viërios isolated from the marine environment. The study also included, the characterization of the virulence or virulence associated gene as most of these Viërios have specific virulence factors such as capsules, toxins, colonization factors and many are known to harbour yet to be identified virulence molecules.

Many species of Viërio could be isolated from water, crustaceans, molluscs and plankton, sampled from different locations along the coastal areas of Kerala, and Aquaculture farms. The sampling stations included Kasaragode, Vadakara, Chavakkadu, Chettuva, Ponnani, Connolly canal, Kochi, Alappuzha, Aqua farm, Aqua farm where fish kill occurred, Omanappuzha, Neendakara etc.

One hundred and eight strains of Viërios could be isolated, identified and stocked before they were characterized.

Phylogenetic trees were constructed using the neighbour joining methods implemented in the DAMBE (Data Analysis in Molecular Biology and Evolution (Xia 2000) software program. Trees were constructed using nucleotide based evolutionary model TN84 method for estimating genetic distances. Statistical support for branching was estimated using 1000 bootstrap steps and nodes with a bootstrap value of 750 or more were taken to represent nodes with significance.
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All the isolates were compared with respect to their stations of isolation and with respect to the species obtained.

Genetic relatedness of all isolates, based on 16SrRNA gene could be established. Phylogenetic tree method could be employed to find the correct identity of the strains. Maximum numbers of *Vibrio cholerae* were isolated from the two stations, Ernakulam and Alappuzha. The station Connoly canal (Kozhikode) was represented by 13 *Vibrio* isolates, and it was the only station represented by highest species diversity with 6 different species.

All the 108 isolates were tested for antibiotic susceptibility. It was found that ampicillin resistance was exhibited at the highest rate (62.04% were resistant against ampicillin). Chloramphenicol and norfloxacin were the most effective antibiotics in controlling the *Vibrios*. All the strains tested (n=108) were susceptible to both these antibiotics. Nalidixic acid resistance was shown by only 0.93% of the isolates tested. But even this small number indicating resistance to the quinolones is significant. In the current context, this information is both cautionary and alarming. Quinolones, a powerful treatment option which are now being used, may be rendered ineffective in the near future, if this seemingly insignificant value gradually increases to alarming proportions.

Multiple Antibiotic Resistance index was found out for those isolates which were resistant to more than one antibiotic. Only 16% of the tested isolates showed multiple antibiotic resistance pattern. A MAR index of 0.4 was shown by two strains of *Vibrios* - FK5 (*Vibrio alginolyticus*) and P6 (*Vibrio harveyi*). These strains were mostly resistant to β-lactams, tetracycline, trimethoprim, nitrofuran and quinolone. A MAR index of 0.3 was show by five strains i.e. FK4, MUS9, CI, P5 and CHAVA4.
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(i). Within the MAR isolates, three belonged to the station Alappuzha and Ernakulam (ALP (VC)5, ALP(VC)2 and EKM10), from where the maximum numbers of *Vibrio cholerae* was isolated. These strains have a MAR index of 0.2. Station Ponnani was represented by three MAR strains i.e., P4, P6 and P8, of which P6 (*Vibrio harveyi*) had the highest MAR of 0.4, which was also observed in strain FK5 (*Vibrio parahaemolyticus*) isolated from the aquafarm where fish kill occurred.

Polymerase Chain Reaction was employed as a rapid screening method to check for the presence of selected virulence genes among the 108 strains of *Vibrios*. Most of the virulence genes tested were absent in the tested *Vibrio* isolates, except for the genes from integrons (*qacEΔ1, Sull and inCS*), *ompw* (a marker gene for *V. cholerae*), *toxR* (the transcriptional regulator of most virulence cascades in *Vibrios*) and *sxt*, an integrative conjugative element. Primers targeting genes *tcpA*, *ace*, *zot* and *ctxA* did not give any positive amplification in any of the *Vibrio* isolates.

A rare and unusual event of lateral gene transfer could be reported, between *V. cholerae* and *V. alginolyticus*. SXT, an integrative conjugative element, which was previously reported only from *Vibrio cholerae*, was found in a non cholera *Vibrio* in this study. Strain FK4, identified as *Vibrio alginolyticus* was found to harbour this element. Conjugation experiments helped to prove that this element could be transferred to *Escherichia coli* HB101 along with its resistance markers, confirming its presence.

Suckling mouse assay was performed with 21 strains to observe the expression of other uncharacterized gastric toxin/toxins, which may express in a susceptible host. More than 50% of the strains tested showed the presence of gastric fluid accumulating type toxin/toxins. Out of eleven *Vibrio* species that showed gastric
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fluid accumulation and a F/A ratio of ≥ 0.09, *V. parahaemolyticus* and *V. alginolyticus* had the maximum representation with three strains each. (Strains CHV3 (1), CHAVA3 and CHAVA4 (4) - *V. parahaemolyticus* and strains CHV2(2), CHV4(3) and FK4 - *V. alginolyticus*).

Conclusion

A rare horizontal gene transfer event could be traced. The movement of the SXT element among the *Vibrionaceae* could be followed. This element was first reported from *Vibrio cholerae* and in this study the same could be confirmed in *Vibrio alginolyticus*. Events such as these, which take place with respect to other virulence/virulence associated genes, may lead to the emergence of pathogenic strains from hitherto non-pathogens or may even give rise to new pathogens.

The results generated in the course of this study paves way for further characterization and detailed study, especially with respect to those strains which showed gastric fluid accumulation in the *in vivo* suckling mouse assay. Antibiotic resistance pattern shown by a sample population of *Vibrios* can be used for deciding treatment options. There is enough scope for further research on these topics towards generating basic knowledge, which can be of immense significance in human and aquaculture health.