

SUMMARY

One of the major unsolved problems in cholera epidemiology is the appearance and disappearance of different strains of *Vibrio cholerae* as the causative agent in an outbreak. For example, the El Tor strain which was always considered to be "harmless" suddenly appeared as "the pathogen" in the 7th pandemic of cholera. Another example is the recent appearance of a strain of *V. cholerae*.

In 1992, a major event took place in history of the disease----a strain of *V. cholerae*, which was subsequently shown to be a new non-01 strain, made its appearance in Southern India. These outbreak strains from India and Bangladesh were found to be serologically identical but were non-agglutinable by the antibodies raised against then known 138 serotypes of *V. cholerae*. On the basis of this observation these strains were, therefore, assigned a new serogroup 0139 with the synonym Bengal. As this strain began to move to the neighbouring countries, it evoked the fear of beginning of the 8th pandemic of cholera. Infections due to *V. cholerae* 0139 in an endemic area were mainly restricted to adults----a subset of population which would otherwise be expected to be resistant to classical (01) cholera because of their earlier exposure. It was conjectured that the 0139 strains could cause such devastation as they could evade immune surveillance because of their altered surface antigen structure.

Strangely, however, by December 1993, cases of cholera due to 0139 began to decline and episodes of cholera due to El Tor began to increase. By May 1994, these early emergent 01 El Tor strains established complete dominance over the 0139 strains. But this predominance appeared to be rather short lived as in September 1996,

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V. cholerae 0139 staged a come back once again and within a short time became the dominant strain.

The problem of disappearance and reappearance of strains is difficult to explain and the factors determining the emergence, disappearance, of continued existence of particular clone of *V. cholerae* are not known. In the absence of any knowledge in this area, it was of interest to know whether the reemerged strains are the same as the ones that existed before or they belonged to a new clones. In this thesis this problem was studied.

In this study, epidemic strains of *V. cholerae* were characterized at molecular level. Studies on the genesis of *V. cholerae* 0139 identified probable progenitor strains. It was observed that *V. cholerae* 0139 Synonym Bengal emerged from serogroup O1 strains typified by isolates M01 and M0477.

Studies on O1 El Tor strains, isolated before and after the 0139 outbreak, revealed that all O1 strains, isolated before the epidemic of 0139, belonged to 6a ribotype of standardized ribotyping scheme proposed by Popovic *et al.* (1993). All "after" strains except two belonged to a hitherto undescribed ribotype. All "previous" strains, except one, had two tandemly arranged CTX genetic elements while all after strains had one. It was also observed that CTX genetic element occupied different chromosomal locations in "before" and "after" O1 El Tor strains. We found that "before" strains harboured a "free" RS1 element away from CTX genetic element and lyogenic phage ϕ 0139 (also called K139).

It appeared from this study that though reemerged 0139 strains had an identical ribotype that of previous 0139 strains isolated in 1992, but they have undergone a

genetic flux at the CTX genetic element as the arrangement of CTX element in reemerged 0139 strains was very different from that of the previous 0139 isolates. 0139 strains isolated in 1992 had two copies of tandemly arranged CTX genetic element and two core regions are connected by two RS1 elements. However, the newly emergent 0139 strains had three copies of CTX genetic element present in tandem, with only one copy of RS1 connecting them. This RS1 element which is connecting the core regions in the newly emergent 0139 strains was unconventional in the sense it had lost the conserved *Bgl*III site and gained *Hind*III site.

Even though the reasons for the emergence of 0139 and then the reemergence of 01 and 0139 is not known, it appears that the reemerged 01 and 0139 strains had greater fitness advantage compared to the preexisting strains. *V. cholerae* in its journey from environment to human gut combats different nutrient concentrations besides variations in temperature and acid pH. While some information is available on heat shock response in *V. cholerae*, not much information exists on how *V. cholerae* overcomes the nutrient deprived conditions, besides two preliminary reports. In this context response in starved epidemic strains to thermal stress was examined and compared to that of an 01 El Tor prototype strain MAK757.

Enhanced or *de novo* synthesis of 24 proteins was observed upon carbon starvation in *V. cholerae* MAK757. Out of which three could be identified as DnaK, GroE and DnaJ, the HSPs. Even though no direct correlation can be established, it appeared that HSPs play some role in protecting *V. cholerae* from acid shock.