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Antibiotic sensitivity pattern of environmental *Vibrio cholerae* of South Bengal

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Abstract:

Vibrio cholerae isolates from crab, prawn, pond and river water samples in southern Bengal were isolated for assessing their antibiotic sensitivity pattern. Seven isolates were biochemically confirmed, with three positive for the *ompW* gene. Resistance to ceftriaxone and nalidixic acid was widespread, limiting their clinical utility. Ofloxacin and streptomycin were highly effective, showing complete sensitivity in several isolates. Concerningly, resistance to carbapenems like Meropenem, Imipenem and polymyxins was observed, posing challenges for managing severe infections. The study highlights the need for routine antimicrobial resistance (AMR) surveillance and emphasizes environmental sources as reservoirs of pathogenic, resistant *V. cholerae*. Future research should employ genomic approaches to better understand AMR mechanisms, informing public health strategies and therapeutic interventions in the region.

Key words: *Vibrio cholerae,* southern Bengal, *ompW* gene, resistance, antimicrobial resistance.

1. Introduction:

Cholera is an acute diarrheal infection involving some serogroups of *Vibrio cholerae,* a gram negative, facultative anaerobe and comma shaped bacterium(Finkelstein 1996). The main reservoirs of *V. cholerae* are aquatic sources such as rivers, brackish waters, and estuaries, often in association with copepods or other zooplankton, shellfish, and aquatic plants(Lutz et al. 2013). The strains which can produce cholera toxin (CT) are responsible for this waterborne disease("Website," n.d.) and even in the 21st century, cholera is still a major cause of death in the developing countries(Ilic and Ilic 2023). Despite all the treatments, cholera remains a global threat to public health. According to researchers there are 1.3 to 4.0 million cases of cholera each year with 21,000 to 143,000 deaths worldwide (Ali et al. 2015). From 1 January to 30 June 2024, a cumulative total of 249793 cholera cases and 2137 deaths were reported globally across five WHO regions ("Multi-Country Outbreak of Cholera, External Situation Report #15 - 19 June 2024," n.d.).

Cholera is treated with oral and intravenous rehydration therapy, antibiotics are also administered as part of the treatment regimen to reduce the stool volume, duration of diarrhea caused by *V. cholerae* and volume of rehydration fluid uptake(Leibovici-Weissman et al. 2014). However, in recent years, treatment failures are often seen with the recurrent emergence of antimicrobial resistant *V. cholerae*. Antibiotic resistance resulted as survival mechanism of various Gram-negative pathogens due to inappropriate usage and use of antibiotics as therapeutic prophylactics against pathogens.(Kulková et al. 2014)

Antibiotic resistance is a major cause of threat to the medical community and it is of utmost importance to regularly monitor the bacterial susceptibility and resistance dynamics. It is particularly crucial when dealing with pathogens capable of environmental proliferation and

implicated in widespread (epidemic or pandemic) infections. Southern Bengal, India, with its many rivers and aquaculture activities, creates ideal conditions for *Vibrio* bacteria to grow and spread antibiotic-resistant genes (Mookerjee et al. 2014). However, we know very little about the antibiotic profile of the environmental *Vibrio cholerae*, to what extent they are resistant to antibiotics, or the reasons behind this resistance. Accordingly we have designed our study to focus on antibiotic resistance pattern of *Vibrio cholerae* in few aquatic bodies and Hooghly (branch of Ganga River) river in monsoon season, when the preponderance of the bacterium is higher.

2. Methodology:

Study Area**:** Riverine water and crustacean samples were collected from preselected sites of a century old diarrhea prone region for our study. The samples were collected during monsoon (July and August). Samples were collected every fortnight, to encompass the *Vibrio* load and their antimicrobial sensitivity.

Crab, Prawn and water from various sources were collected and spread on TCBS (Thiosulfate citrate bile sucrose) (Millipore) agar plates and incubated at 37ºC for 18 hours. Microbes from prawn and crab shells were first grown in nutrient broth for enrichment and then spread on TCBS agar. Yellow colonies with elevated centers, indicative of *Vibrio cholerae*, were isolated and cultures were maintained at 4ºC.

Presumptive Vibrio colonies from TCBS agar plates were inoculated onto TSI (Triple Sugar Iron) slants and incubated at 37ºC for 18 hours to biochemically confirm the presence of *Vibrio cholerae*. The presence of *Vibrio cholerae* in the culture can be determined by observing **acid/acid** or **alkali/acid** production.

The biochemically positive *V. cholerae* culture, a loopful of bacteria was streaked on nutrient agar plates. After that “Boil template method” was done for genomic DNA extraction and plasmid was isolated by using a plasmid miniprep kit. PCR assay was performed for the ***ompW* genes** for te molecular confirmation of the *Vibrio cholerae*.

Antibiotic sensitivity test was also done using the **Disc diffusion method by Kirby and Bauer** where different *V. cholerae* cultures were inoculated in NB (Nutrient broth) and incubated at 37ºC. Next step was spreading those cultures on MHA (Mueller Hinton Agar) (Himedia) plates. The antibiotic discs were then carefully placed to the top of the bacterial culture and the plates were incubated at 37ºC for 24 hours approximately. After that the diameter of the zone of inhibition, which is the area around the antibiotic where bacteria can't grow, were measured.

Following antibiotics were used- **MRP 10**- Meropenem, **AMC 30**- Amoxicillin, **CTR 30**- Ceftriaxone, **IPM 10**- Imipenem, **AMP 10**- Ampicillin, **AZM 15**- Azithromycin, **PB 300**- Polymyxin B, **S10**- Streptomycin, **OF2**- Ofloxacin, **NA30**- Nalidixic Acid.

3. Result:

During the study period a total of 8 water samples and 4 crab and 4 prawn samples were collected from the study area during the two months of sampling. A total of 22 suspected Vibrio cholerae were isolated from the samples. 15 presumptive Vibrio cholerae were isolated from the Crab samples, 2 from the prawn samples and only 5 samples were isolated from the water samples.

Out of 22 V. cholerae isolates from the TCBS agar only 7 isolates were confirmed biochemically, showing positive reaction for V. cholerae.

7 isolates of *Vibrio cholerae* (biochemically proved) were analysed for the presence of the *ompW* gene, out of which only 3 isolates were found to harbour the *ompW gene*; one each from crab shell, prawn shell, and river water.

All the 7 biochemically positive V. cholerae were subjcted to the Antibiotic sensitivity test the result is shown in the Table-1

Table1: Antibiotics sensitivity Test

Sample source	Against No. of antibiotics	
	Sensitive	Resistant
Ganga VC	3	4
Pond VC	3	4
Crab VC1	2	7
Crab VC2	5	4
Crab VC3	1	8
Crab VC4	4	5
Prawn VC1	3	6

4. Discussion:

Gangetic south Bengal is a diarrhoea and cholera endemic foci since time immemorial. In our present endeavor *Vibrio cholerae* were detected in water samples of river and pond of this region, as Vibrio are endemic to this region and are originated in the estuarine and brackish water systems and then carried to the upper reaches of the river by tidal flow.

The isolation rate of *Vibrio cholerae* was highest form the crab samples followed by the Prawn samples, showing a clear preference (77% isolates) of the *V cholerae* for the chitin as carbon source and thus the chitinous organisms like crabs and prawns. The preponderance of *V. cholerae* in the water is only 23% of the isolates. Thus chitinous exoskeleton harbors higher number of V. cholerae than the other sources.

The biochemical identification also shows the higher number of biochemically positive V. cholerae isolated from the crabs (4/7) and prwans (1/7). Interestingly only 3 isolates were found to harbour ompW gene, with one sample each from crab, prawn and water.

All 7 biochemically positive Vibrio isolates were subjected to antibiotic sensitivity test, where it was found that Meropenem, Imipenem and Ofloxacin were mostly sensitive among most of the isolates as these are the higher generation antibiotics. But two V. cholerae isolate one from Crab was resistant to meropenem and imipenem, and one from prawn is resistant to imipenem which is very alarming, as resistance to these strong antibiotics in the environmental bacteria increases manyfold the chances of the spread of this resistance in other groups of bacteria and render antibiotics ineffective. All the 7 isolates were resistant to Ceftriaxone, and 6 isolates were resistant to Nalidixic acid, this shows the potential challenge we are up to with the use of the antibiotics. Other antibiotics also shows mixed sensitivity.

In the south Bengal community settings where the community comes very close to the natural water bodies and many people rely on these water sources for their daily needs and where shelled crustacean foods are a significant part of the local diet, the presence of these harmful bacteria like V. cholerae in itself poses a major public health risk. Additionally, the antimicrobial-resistant of these bacterial strains in water, crab, and prawn samples adds to the health risk.

Thus, our study hints at the potential threat of the community getting exposed to these harmful bacteria and also the present condition of the antibiotic resistance of these environmental bacteria to the commonly used drugs. Our study also suggests regular survey to keep a vigilant on the antimicrobial resistance AMR in different environmental sources to curb the menace of diarrhea/ cholera in the community settings.

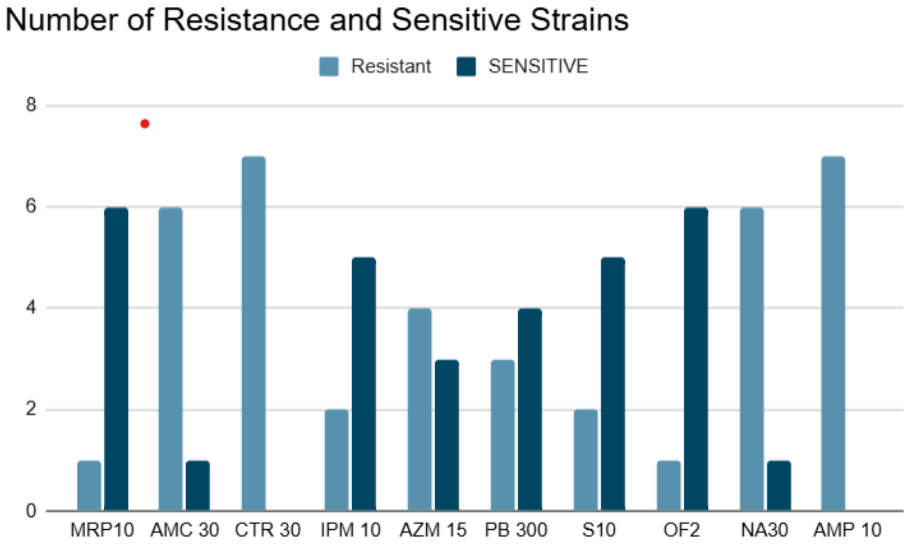

Fig: 1 Chart of antibiotic resistance

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