RESEARCH ARTICLE

Antibiogram of *Vibrio parahaemolyticus* Isolated from Fish and Prawn in and around Navsari, Gujarat

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Abstract

Food-borne *Vibrio parahaemolyticus* has global economic and public health concern. The frequent way for humans to contract these bacteria is through seafood. In this study, 350 samples were analysed for the presence of *Vibrio parahaemolyticus*. Of those, 40 samples were found positive for the bacteria. Ten different antibiotics were used to test the antibiotic sensitivity of these 40 isolates, and they exhibited variable susceptibility pattern. The isolates showed 100% resistance to penicillin G followed by 80% and 75% resistance to ampicillin and streptomycin, respectively. The isolates were highly sensitive to ciprofloxacin (97.5%), followed by trimethoprim (90%), gentamicin (90%), chloramphenicol (87.5%), amikacin (80%), tetracycline (65%) and cephalothin (65%). Of the 40 isolates, 45.0%, 22.5%, 5.0%, 7.5% isolates were found resistant to 3, 4, 5 and 6 antibiotics, respectively. Multiple antibiotic resistance (MAR) index value above 0.2 was shown by 80% of the isolates. The presence of MAR highlights the essential need to determine drug susceptibility and monitor antimicrobial resistance profiles. This is crucial for enhancing food safety and protecting public health. By identifying resistant strains, we can develop more effective treatment strategies and implement measures to control the spread of resistant pathogens, thus preserving antibiotic efficacy and ensuring public well-being.

Key words: Antibiogram, Fish, MAR index, Prawn, *Vibrio parahaemolyticus*.

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Introduction

Vibrio parahaemolyticus is a halophilic, gram negative, oxidase positive, motile, straight or curved rod-shaped and facultative anaerobic microorganism.This bacterium is a natural component of aquatic habitats of brackish or marine environments with varied salinity. It is classified as moderately halophilic and can survive and multiply at concentrations of 1 to 9% sodium chloride (NaCl), with 3% NaCl providing the best growth conditions (Whitaker *et al.,* 2010; Kalburge*et al.,* 2014). *V. parahaemolyticus* is regarded as an emerging species due to its participation in outbreaks following the ingestion of contaminated food, particularly partially cooked fish and shellfish. In human beings, *V. parahaemolyticus* causes acute gastroenteritis, Occasional cases of bloody diarrhoea with distinctive, reddish-watery stools called "Meat Washed" diarrhoea are recorded. Within 4-96 h of consuming the contaminated food, symptoms of disease are noticed and may continue for up to 3 days.

The aquaculture industry frequently relies on antimicrobials for treating infectious diseases. However, the widespread use of these agents has contributed to the emergence of antimicrobial resistance (AMR) among pathogens, rendering many antimicrobials ineffective. Furthermore, inappropriate antibiotic usage in aquaculture leads to antibiotic residues in aquatic species like fish in addition to increasing the selection of antibiotic-resistant bacteria and the spread of antibiotic-resistant genes (Miranda *et al.,* 2018). Most strains of *V. parahaemolyticus* that were isolated from clinical and environmental samples showed a high level of resistance to several antibiotics, including colistin, tobramycin, amoxicillin, ampicillin, carbenicillin, cefazolin, ceftazidime, cephalothin,cefazolin, tetracycline, streptomycin, kanamycin and ciprofloxacin (Devi *et al.,* 2009; Yano *et al.,* 2011; Raissy*et al.,* 2012). A developing public health problem that is of great concern to many nations and sectors is antibiotic resistance among a wide spectrum of pathogens

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(WHO, 2014). So, this study was set out to ascertain the antibiotic resistance pattern of *V. parahaemolyticus* that were isolated from fish and prawn in and around Navsari, Gujarat.

Materials and Methods

Bacterial Isolates

A thorough bacteriological analysis of 350 samples (150-Fish, 150-Prawn, 30 samples of hand swabs and 20 stool samples from fish handlers) were processed following standard protocol, i.e., *Bacteriological Analytical Manual* (BAM), U.S. Food and Drug Administration(USFDA) method (Kaysner et al., 2004).The fish and prawn samples were triturated to get homogenate. Later on, the homogenate as well as human swab and stool samples collected aseptically were enriched in Alkaline Peptone Water (APW) and incubated for 24 h at 37°C. On next day secondary enrichment was carried out using Salt Polymyxin B Broth (SPB) at 37°C for 24 h. The enriched samples were then plated on Thiosulfate Citrate Bile Salts Sucrose agar (TCBS) and *Vibrio Parahaemolyticus* Sucrose Agar (VPSA). The *V. Parahaemolyticus* isolates exhibited characteristic green color colonies and bluish green colonies on TCBS and VPSA, respectively. These colonies were sub-cultured on TSA (Tryptone soya agar) and the pure colonies were stored in 20% sterile glycerol under -20°C for further experiments.

Antibiogram

The isolates were subjected to antibiotic sensitivity test on Muller-Hinton agar (2% NaCl) following CLSI, 2017 guidelines. The 10 different standard antibiotics discs used in the present study fell into 4 categories, I. Cell wall synthesis inhibitors (penicillin G, ampicillin, cephalothin), II. Protein synthesis inhibitors (amikacin, chloramphenicol, gentamicin, streptomycin and tetracycline), III. DNA gyrase inhibitors (ciprofloxacin), and IV. Antifolate antibiotics (trimethoprim).

The Muller Hinton broth was inoculated with 3-5 colonies and incubated at 37°C for 2-8 h until achieving a turbidity equivalent to 0.5 on the Mac Farland scale to have a final concentration of 1.5 x10^8 CFU/mL. Subsequently, a sterile swab was picked, pressed up against the tube well to drain any extra liquid and then seeded, rotating at least twice on the surface of the MHA plates. The MHA plates were kept for five minutes. With the sterile forceps 10 antibiotic discs were placed at equal distances from each other. Then the plates were inverted and incubated at 37°C for 18 h. The zone of inhibition was measured with the aid of a ruler. The interpretation was made as per the zone size interpretation chart provided by the manufacturer of discs (HiMedia Lab Pvt. Ltd., Mumbai, India).

Multiple Antibiotic Resistant (MAR) = a/b Where, a - represents number of antibiotics, resistant to the isolate, and b - represents total number of antibiotics tested against the isolate. If, MAR is > 0.2 it means the antibiotic is resistant to the isolate, whereas MAR ≦ 0.2 indicates that the antibiotic is sensitive (Krumperman, 1983).

Statistical Analysis

The IBM® SPSS® software (version 20.0) was used to perform the chi-square test to the collected data in order to analyse the antibiotic sensitivity pattern. The one way ANOVA and Duncan's Multiple Range test were used to determine differences between the means at 5% and 1% level of significance.

Results and Discussion

Out of total 350 samples, 40 (11.42%) samples were positive for *V. parahaemolyticus*. Antibacterial sensitivity testing was performed on all 40 isolates using 10 antibiotics and they exhibited variable susceptibility pattern.*V. parahaemolyticus* isolates were highly sensitive to ciprofloxacin (97.5%), followed by trimethoprim (90%), gentamicin (90%), chloramphenicol (87.5%), amikacin (80%), tetracycline (65%) and cephalothin (65%). An intermediate sensitivity of 22.5% and 20.0 % was exerted against cephalothin, and streptomycin, respectively while others showed lower %intermediate sensitivity. The penicillin G, ampicillin, streptomycin had

Table 1: Summary of the antibiotic susceptibility test of *V. parahaemolyticus* isolates (n=40)

Sr.No	Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
1	Penicillin G	-	-	40 (100)
2	Ampicillin	05 (12.5)	3 (7.5)	32 (80)
3	Cephalothin	26 (65)	9 (22.5)	05 (12.5)
4	Amikacin	32 (80)	2 (5)	06 (15)
5	Chloramphenicol	35 (87.5)	4 (10)	01 (2.5)
6	Gentamicin	36 (90)	-	04 (10)
7	Streptomycin	02 (5)	8 (20)	30 (75)
8	Tetracycline	26 (65)	3 (7.5)	11 (27.5)
9	Ciprofloxacin	39 (97.5)	1 (2.5)	-
10	Trimethoprim	36 (90)	-	04 (10)

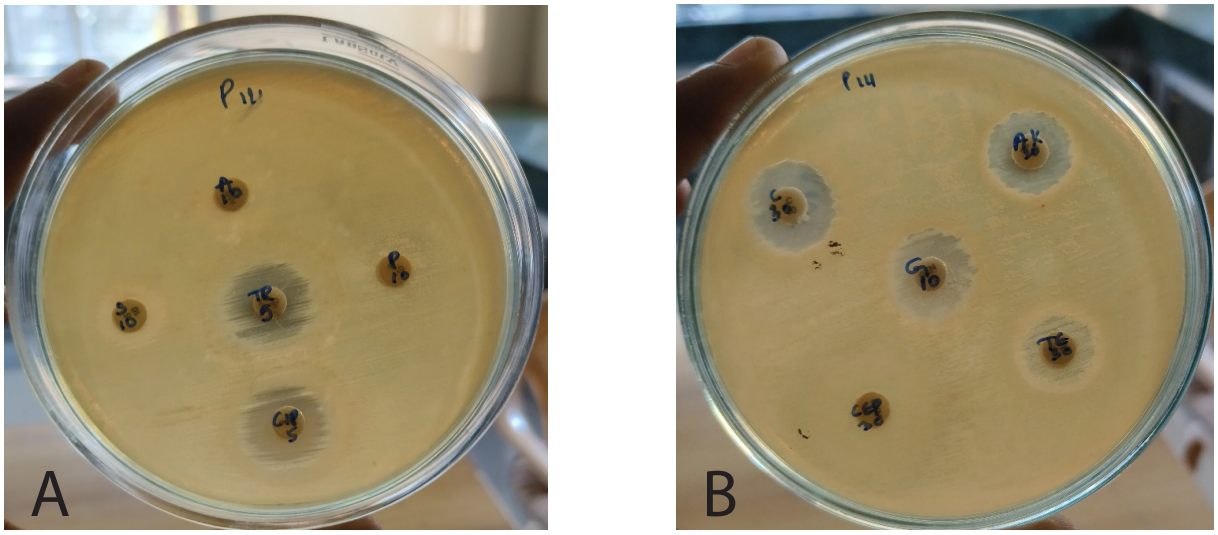

Fig. 1: Anti-microbial susceptibility patterns of *Vibrio parahaemolyticus* showing zones of inhibition of (A) Ampicillin, Penicillin, Streptomycin, Trimethoprim, Ciprofloxacin, and (B) Chloramphenicol, Amikacin, Gentamicin, Tetracycline, Cephalothin

shown the resistance of 100%, 80%, 75%, respectively on *V. parahaemolyticus* isolates (Fig. 1; Table 1).

The present finding of 100% resistance of *V. parahaemolyticus* to penicillin-G was in agreement with the results of Kagiko *et al.* (2001), Quintoil *et al.* (2007), Patel *et al.* (2018). On the contrary, Kshirsagar *et al.* (2013) showed 77.77% resistance on *V. parahaemolyticus* isolates, which might be due to more frequent use of such antibiotics in the fisheries sector over the years. In the present study 80% resistance, 7.5% intermediate resistance and 12.5% sensitivity was exhibited by *V. parahaemolyticus* isolates to ampicillin. The results indicating resistance simulated to the findings of Chakraborty *et al.* (2008), Ghenem and Elhadi (2018) and Narayanan *et al.* (2020), who showed 80.9%, 88.0% and 79.3% resistance, respectively. On the contrary, El-zamkan*et al.* (2023) showed 100 % resistant to ampicillin, which was quite different than the present study and warning sign of further use of this antibiotic in the area under the study.

The sensitivity of *V. parahaemolyticus* isolates towards cephalothin was 65%, intermediate resistance of 22.5% and resistance of 12.5%, which was in close proximity of the findings of Kshirsagar *et al.* (2013), who reported 66.66%, 22.22%, 11.11% of sensitivity, intermediate resistance, resistance, respectively. On contrary, Ghenem and Elhadi (2018) reported 76% resistance, 15% intermediate resistance, 10 % sensitivity towards cephalothin antibiotic.

The 80% isolates of *V. parahaemolyticus* showed sensitivity to amikacin, 15% and 5% isolates showed resistance and intermediate resistance, which was in approximation to the findings of Patel *et al.* (2018) and Elhadi *et al.* (2022). However, data differed from observation made by Ghenem and Elhadi (2018), who showed 59% sensitivity, 29% intermediate resistance and 12% resistance. This could be due to change in the pattern of use of amikacin in the area under survey.

The chloramphenicol expressed 87.5% sensitivity, 10% intermediate resistance and 2.5% resistance in the present investigation. The present sensitivity observed was in accordance with Chakraborty *et al.* (2008), Kshirsagar *et al.* (2013) Patel *et al.* (2018) and Elhadi *et al.* (2022)., El-zamkan *et al.* (2023) reported 81.8% sensitivity, 18.2% intermediate resistance. On other hand cent percent sensitivity to chloramphenicol was recorded by Biswarup (2014), Ghenem and Elhadi (2018) and Narayanan *et al.* (2020).

V. parahaemolyticus isolates showed 90% sensitivity and 10% resistance to gentamicin in the present study, which was similar to the findings of Elhadi *et al.* (2022), while as high as 100% sensitivity was observed by Biswarup (2014), Ghenem and Elhadi (2018), Patel *et al.* (2018) and Narayanan *et al.* (2020). However, lower sensitivity of varying levels (38% to 80%) was reported by others (Chakraborty *et al.,* 2008; Kshirsagar *et al.,* 2013; Parthasarathy *et al.,* 2021; El-zamkan *et al.* (2023). The difference noted might be due to variation in the use of gentamicin over the period of time.

In the present study, isolates exhibited 75% resistance, 20 % intermediate resistance, 5% sensitivity to streptomycin, which concurred with Chakraborty *et al.* (2008) and Patel *et al.* (2018), who showed 76.2% and 71.42% resistance, and Ghenem and Elhadi (2018), who reported 5% sensitivity, but contrary to the finding of Kagiko *et al.* (2001), who recorded 100% sensitivity.

The 65% isolates were sensitive and 27.5 % isolates were resistant to tetracycline in the present study, whereas Chakraborty *et al.* (2008), Ghenem and Elhadi (2018) and Narayanan *et al.* (2020) showed almost 100% sensitivity to tetracycline, while El-zamkan *et al.* (2023) showed 81.8% sensitivity and 18.2% resistance to tetracycline. On the contrary, others (Kshirsagar *et al.,* 2013; Patel *et al.*, 2018; Parthasarathy *et al.*, 2021) showed 50 to 72% resistance of *V. parahaemolyticus* towards tetracycline.

The sensitivity of *V. parahaemolyticus* isolates towards ciprofloxacin was 97.5% and 2.5% was intermediate resistance in the present study. These findings concurred well with Biswarup (2014), Patel *et al.* (2018), Narayanan *et al.* (2020) and El-zamkan *et al.* (2023), who reported 97-100% sensitivity, while Ghenem and Elhadi (2018) showed 80% sensitivity towards ciprofloxacin, which was lower than the present study.

In the present study, 90% isolates exhibited sensitivity and 10% isolates were resistant to trimethoprim, which was similar to the results of Chakraborty *et al.* (2008) and Parthasarathy *et al.* (2021). However, Ghenem and Elhadi (2018), Narayanan *et al.* (2020), Elhadi *et al.* (2022) showed cent percent susceptibility of *V. parahaemolyticus* isolates to trimethoprim, while Patel *et al.* (2018) reported 85.71% sensitivity. In general, the difference noted might be due to variation in the use of particular antibiotics over the period of time in the area.

The comparative analysis between inhibition zone by different antibiotics revealed descending order of inhibition zone (sensitivity) as Ciprofloxacin> Chloramphenicol> Amikacin> Gentamicin> Trimethoprim> Cephalothin> Tetracycline> Ampicillin> Streptomycin> Penicillin-G (Table 2). Comparison for inhibition zone at 5% as well as 1% significance level revealed that inhibition zone of ciprofloxacin was significantly highest and that of penicillin-G was significantly lowest. Inhibition zones of chloramphenicol, amikacin, gentamicin, trimethoprim, cephalothin andtetracycline did not differ significantly among themselves, but were significantly higher than ampicillin and streptomycin. Inhibition zone of ampicillin was higher than that of streptomycin at 5% significance, but not significantly different at 1% significance. Inhibition zones of ampicillin and streptomycin were significantly higher than that of penicillin-G both at 5% and 1%.

Comparative analysis between inhibition zone by different antibiotic mechanisms revealed descending order of sensitivity as DNA gyrase inhibitors> Antifolate antibiotics> Protein synthesis inhibitors> Cell wall synthesis inhibitors. Comparison at 5% and 1% significance level revealed that sensitivity of DNA gyrase inhibitors was significantly higher and that of Cell wall synthesis inhibitors was significantly lower, however, Antifolate antibiotics and Protein synthesis inhibitors did not differ significantly.

MAR (Multiple Antibiotic Resistance)

The MAR index was computed for isolates of *V. parahaemolyticus* as described by Krumperman (1983). From Table 3 and Table 4, it is evident that 45.0%, 22.5%, 5.0%, 7.5% isolates were found resistant to 3, 4, 5 and 6 antibiotics. In the present study, the antibiogram indicated that 80% *V. parahaemolyticus* isolates showed significant MAR index values above 0.2, indicating high danger of such bacteria to human being expressing greater risk of contamination (Baliga *et al.,* 2019; Tan *et al.,* 2020).

Tan *et al.* (2020) challenged 120 *V. parahaemolyticus* isolates recovered to 24 antibiotics. Each isolate was discovered to have a unique antibiotic resistance profile, with MAR indices ranging from 0.04 to 0.71. The BC4 isolate had the highest MAR value (0.71) and was resistant to 17 drugs. Most isolates showed resistance to three or more antibiotics. Elhadi *et al.* (2022) reported the MAR index above 0.2 in 30% of isolates, *i.e.,* 30% expressed multi-drug resistance, which was much lower than the results in the present study and it reflects the more judicious use of antibiotics in the area surveyed. El-zamkan *et al.* (2023) reported MAR index ranging from 0.16 to 0.5 for *Vibrio* spp.which was recovered from raw milk, dairy products and water.

The misuse of antibiotics in fisheries and aquaculture practice causes MAR in *V. parahaemolyticus*. The availability of such antibiotics is crucial for food security and production, but improper use negates their advantages. Furthermore, the discharge of sewage which contains antibiotics is also contaminating water bodies, which make harmful microorganisms more resistant (Xu *et al.,* 2016). MDR gene transmission has the potential to compromise the effectiveness of antibiotics used to treat vibrionic infections. The resistance gene also makes treating severe vibrionic infections more difficult (Liu *et al.,* 2013). Additionally, drug resistance genes found in marine environments may serve as potential reservoirs for the horizontal gene transfer of

Table 2: Zone diameter (Mean ±SE) expressed by different antibiotics as per mechanism of action on *V. parahaemolyticus* isolates

Mechanism	Inhibition zone (mm)	Antibiotic	Inhibition zone (mm)
Cell wall synthesis inhibitors (n=120)	10.48^{cC}±0.74	Penicillin-G	0.35^{fD}±0.35
		Ampicillin	12.68^{dC}±0.54
Protein synthesis inhibitors (n=200)	17.44^{bB}±0.34	Cephalothin	18.40^{bcB}±0.53
		Amikacin	19.38^{bcB}±0.53
		Chloramphenicol	19.88^{bB}±0.45
		Gentamicin	19.20^{bcB}±0.60
		Streptomycin	10.93^{eC}±0.52
		Tetracycline	17.83^{cB}±0.65
DNA gyrase inhibitors (n=40)	22.30^{aA}±0.19	Ciprofloxacin	22.30^{aA}±0.19
Antifolate antibiotics (n=40)	18.78^{bB}±0.60	Trimethoprim	18.78^{bcB}±0.60

The superscripts across columns differ significantly for lower case (a,b, …..) at 5% p≤0.05 and for upper case (A, B …..) at 1% p≤0.01.

such genes to pathogenic bacteria through conjunction, transformation or transduction (Silvester *et al.,* 2015).

Table 3: Summaryof MAR index of the *V. parahaemolyticus* isolates from fish

Sr. No	No of resistant antibiotics	MAR value	No. of isolates
1	1	0.1	1 (2.5 %)
2	2	0.2	7 (17.5 %)
3	3	0.3	18 (45 %)
4	4	0.4	9 (22.5 %)
5	5	0.5	2 (5 %)
6	6	0.6	3 (7.5 %)

Table 4: Summary of MAR value and antibiotic resistant pattern of *V. parahaemolyticus* isolates

S. No	Sample No	Resistant patterns	MAR index
1	MF-2	P, AK, S	0.3
2	MF-5	P, A	0.2
3	MF-6	P, A, S	0.3
4	MF-13	P, A, S, TE	0.4
5	MF-21	P, A,	0.2
6	MF-27	P, A, S	0.3
7	MF-35	P, A, S, TE, TR	0.5
8	MF-39	P, GEN	0.2
9	MF-44	P, A, CEP, S	0.4
10	MF-50	P, A,	0.2
11	MF-54	P, A, AK, C, S, TE	0.6
12	MF-61	P, S, TE	0.3
13	MF-68	P, A, S,	0.3
14	MF-73	P, AK, S, TE	0.4
15	MF-79	P, A, GEN	0.3
16	MF-82	P, A, GEN, S, TE, TR	0.6
17	MF-85	P, A, S	0.3
18	MF-91	P, A, S	0.3
19	MP-3	P, A, S	0.3
20	MP-7	P, A, CEP	0.3
21	MP-17	P, AK, S	0.3
22	MP-23	P, A, S, TE	0.4
23	MP-38	P, A, S	0.3
24	MP-42	P, A	0.2
25	MP-47	P, A, S, TE	0.4
26	MP-55	P, A, CEP, S	0.4
27	MP-59	P, A, S	0.3
28	MP-66	P	0.1
29	MP-69	P, A, S	0.3
30	MP-74	P, A, AK, S	0.4
31	FF-6	P, A, S, TE	0.4
32	FF-25	P, A, GEN	0.3
33	FF-48	P, A, S	0.3
34	FF-54	P, A, CEP, S, TR	0.5
35	FP-16	P, S	0.2
36	FP-35	P, A, S, TE	0.4
37	FP-69	P, A,	0.2
38	HS-9	P, A, S	0.3
39	HS-17	P, A, CEP, AK, S, TE	0.6
40	HS-23	P, S, TR	0.3

Conclusion

The findings of present study revealed sensitivity of *V. parahaemolyticus* isolates from fish in descending order to 10 antibiotics tested as Ciprofloxacin> Chloramphenicol> Amikacin> Gentamicin> Trimethoprim> Cephalothin> Tetracycline> Ampicillin> Streptomycin> Penicillin-G. DNA gyrase inhibitors were more effective than Antifolate antibiotics followed by Protein synthesis inhibitors and Cell wall synthesis inhibitors. 80% of the isolates had MAR index values greater than 0.2, suggesting that the aquatic environment in the sampling location may be impacted by and polluted with antibiotics from both human and animal sources. For the purpose of enhancing and ensuring food safety and public health, MAR assertion is crucial in assessing medication susceptibility and tracking the profile of antimicrobial resistance.

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