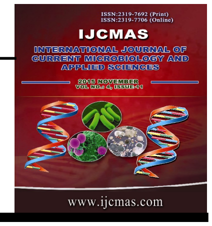
Int.J.Curr.Microbiol.App.Sci (2015) 4(11): 523-535



International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 11 (2015) pp. 523-535**
http://www.ijcmas.com

Original Research Article

Multiple Antibiotic Resistance pattern of *Vibrio harveyi* from Luminous Vibriosis affected cultured Tiger Shrimp, *Penaeus monodon* in Andhra Pradesh, India

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Keywords

Multiple antibiotic resistance, *Vibrio harveyi*, *P. monodon*, Culture ponds

A B S T R A C T

An investigation was undertaken to determine the extent of antibiotic resistance exhibited by *Vibrio harveyi*, isolated from diseased *Penaeus monodon*, collected from culture ponds located in East Godavari District of Andhra Pradesh. A total of 159 isolates of *Vibrio harveyi*, of which 110 are from four Modified-Extensive ponds (ME1, ME2, ME3, ME4) and 49 are from four Semi-Intensive ponds (SIA, SIB, SIC, SID), were screened for their susceptibility to 22 antibiotics. All the isolates from ME and SI ponds were resistant to penicillin G and 100% susceptibility was observed in the case of all the isolates of ME ponds towards Ciprofloxacin and Norfloxacin. The isolates from ME ponds were more resistant than those from SI ponds and the isolates of ME 1 pond exhibited 100% resistance towards eight antibiotics, whereas those of SI ponds were 100% resistant to only one antibiotic. A significant difference was observed in the Multiple Antibiotic Resistance (MAR) of the isolates from ME ponds, whereas the difference was not significant in the case of isolates from SI ponds. MAR against 4 - 10 antibiotics was highest in the isolates from pond ME 4 (90.5%), followed by those from ME 3 (76.7%) and ME 2 (11.4%) and it was highest with 40 % in the isolates from SI D pond. The study revealed the occurrence of highly virulent strains of *Vibrio harveyi* in shrimp culture ponds, which developed resistance to many antibiotics due to indiscriminate usage of antibiotics.

Introduction

Luminous vibriosis is a serious disease problem in culture ponds and hatcheries of shrimp, all over the world, causing mass mortalities. Especially, *V. harveyi*, isolated from shrimp larvae as well as from juveniles and adult shrimps from culture ponds, has been identified as the most pathogenic species (Karunasagar *et al.,* 1994; Jiravanichpaisal *et al.*, 1994; Abraham *et al.*, 1995, 1997; Lavilla-Pitago *et al.,* 1990, 1992, 1998; Otta *et al.*, 1999; Tendencia *et al.*, 2001; Sengupta *et al.*, 2003; Jayasree *et al.,* 2006, 2008). To control the luminous vibriosis, several antibiotics have been tried in hatcheries as well as culture systems of shrimp. Extensive work has also been carried out on the usefulness of antibiotics in controlling diseases in culture ponds and hatcheries (Abraham *et al.,* 1997; Sengupta *et al.,* 2003; Anderson *et al.,* 1999; Mohney *et al.,* 1992; Ruangpan and Kitao, 1992; Hameed *et al.*, 1995; Abraham *et al*., 1997; Moriarity, 1998). Usage of antimicrobials in aquaculture, started with sulphanamides (Gutsell, 1946) followed by chloramphenicol, oxytetracycline (OTC), kanamycin, nitrofurazone, oxolonic acid and sodium nifurstyrenate, flumequin, ciprofloxacin (Austin and Austin, 2003). Indiscriminate use of chemicals and drugs, however, has lead to the development of multiple antibiotic resistance in organisms against these drugs, which may ultimately affect the cultured shrimp and environment. (Tendencia *et al.,* 2001; Uma *et al.,* 2003; Orozoa *et al.*, 2008; Abraham *et al.*, 2009). Transfer of resistant genes to non target organisms, including man, has also been envisaged.

Along the coast of Andhra Pradesh shrimp culture is practiced on a large scale. Apart from viral diseases, luminous vibriosis is also identified as a major problem which is adversely affecting the performance of both hatcheries and growout ponds. Although antibiotics have been used extensively to control luminous vibriosis, so far no information is available from Andhra Pradesh, on the susceptibility and antibiotic resistance of luminous *Vibrio* spp. to various antibiotics, a knowledge of which is useful to prevent epizootics due to luminous vibriosis. The present study compares the antibiotic sensitivity and resistance pattern of luminous *Vibrio harveyi* isolates from diseased shrimp in Modified Extensive (ME) and Semi Intensive (SI) culture systems of East Godavari District of Andhra Pradesh.

Materials and Methods

Shrimp affected by luminous vibriosis were collected from four Modified extensive ponds (designated as ME1, ME2, ME3, ME4) and four Semi-intensive ponds (designated as SI A, SI B, SI C, SI D) located in East Godavari District of Andhra Pradesh (India) during the period 2009–2011.

A total of 159 isolates of *V. harveyi* from the luminous vibriosis affected shrimp, of which 110 are from four ME ponds (i.e., 24 from ME 1, 35 from ME 2, 30 from ME 3 and 21 from ME4) and 49 are from four SI ponds (i.e. 15 from SI A, 12 each from SI B,C and 10 from SI D), were screened for their susceptibility towards 22 antibiotics (commercially available discs from Himedia, Mumbai) by agar disc diffusion method on Muller Hinton Agar (MHA) supplemented with 1% NaCl. The antibiotic impregnated discs (Himedia, Mumbai) used include amoxicillin, ampicillin, cephadroxil, cefazolin, chloramphenicol, ciprofloxacin, clotrimazole, cloxacillin, co-trimoxazole, erythromycin, furazolidin, gentamycin, metronidazole, nitrofurazone, norfloxacin, oxytetracycline, pefloxacin, penicillin – G, rifampacin, streptomycin, tetracycline and trimethoprim. The MHA plates were inoculated with 24 h. luminous *Vibrio* cultures and the antibiotic discs (commercially available from Himedia, Mumbai) were placed on the agar plates with the cultures at equal distances and incubated for 24 hr. at 30°C. After incubation, the diameter of the zone of inhibition was measured by using Kirby Bauer Scale and is compared with zone diameter interpretative chart, to determine the sensitivity of the isolates.

Antibiotic resistance profile

The resistance profile and resistance pattern were determined from the antibiogram data (Krumperman, 1983). The Multiple Antibiotic Resistance (MAR) was attributed to an isolate if it showed resistance to more than 3 antibiotics. The multiple antibiotic resistance index (MARI) for a single isolate is determined by calculating a/b value, where "a" represents the number of antibiotics to which the isolate was resistant and "b" represents the number of antibiotics to which the isolate was exposed. The significance of the difference in the Multiple Antibiotic Resistance patterns among the isolates from various culture systems and sources was assessed by ANOVA test. The extent of similarity between ponds in the antibiotic sensitivity pattern was determined by calculating Jacquard's coefficient of similarity.

Results and Discussion

Results of the susceptibility of *Vibrio* isolates tested against 22 antibiotics were presented in tables 1–3.

ME Ponds

All the 110 isolates (100%) from the four ME ponds showed susceptibility to two antibiotics viz., ciprofloxacin and norfloxacin and also found resistant to the drug, penicillin G (Figure 1).

In ME1 pond, the isolates were susceptible to only four antibiotics and 100% of isolates exhibited sensitivity to ciprofloxacin and norfloxacin, followed by erythromycin (41.7%) and oxytetracycline (21.8%). The number of antibiotics to which the isolates were susceptible varied from pond to pond, 4 in the case of ME 1, 8 in ME2, 10 in ME 3 and 11 in ME 4. Apart from norfloxacin and ciprofloxacin, the isolates from ME 4 pond showed 100% susceptibility to oxytetracycline and tetracycline. Out of all the four ME ponds, the isolates of ME 4 exhibited more susceptibility compared to the other three ponds. ANOVA revealed significant variation in the performance of antibiotic susceptibility between and within ME ponds ($P < 0.5$).

All the isolates of ME 1 pond exhibited resistance towards 8 antibiotic drugs viz., chloramphenicol, cephazolin, metronidazole, nitrofurazone and penicillin G. In ME 2 pond, 100% resistance was observed towards 6 antibiotics, viz, chloramphenicol, clotrimazole, cloxacillin, metronidazole, nitrofurazone and penicillin - G. 100% isolates of ME 3 pond were resistant towards three antibiotic drugs viz., metronidazole, nitrofurazone and penicillin - G respectively. ANOVA revealed significant variation in the antibiotic resistance between and within ME ponds ($P < 0.5$).

SI Ponds

The susceptibility of *Vibrio* isolates of SI ponds towards 22 antibiotic drugs showed wide variation (Figure 2). In SI A pond, maximum susceptibility of the isolates was exhibited towards amoxycillin (80 %), followed by chloramphenicol (73.4%). The isolates of SI B exhibited maximum sensitivity (91.7%) towards oxytetracycline. In SI C pond, the *Vibrio* isolates showed 100% sensitivity towards ciprofloxacin followed by norfloxacin (91.7%). The isolates of *vibrio* from SI D pond, showed 100% susceptibility to ampicillin. 100% isolates of all the SI ponds were resistant to the drug penicillin – G.

When compared to the isolates of SI ponds, isolates of ME ponds exhibited more resistance towards the antibiotics. Within SI ponds, all the antibiotics were found to be effective but the susceptibility of the isolates varied from pond to pond and also among the antibiotics. ANOVA revealed significant difference in the antibiotic susceptibility of isolates between and within SI ponds ($P<0.05$).

Multiple antibiotic resistance

The pattern of multiple antibiotic resistance in Vibrios from different ponds is represented in figure 3.

ME ponds

In ME 1, 41.7% isolates exhibited resistance against 16 antibiotics and the Multiple Antibiotic Resistance (MAR) Index of 24 isolates of ME 1 ranged from 0.6 to 0.7 and from 0.4 to 0.6 in ME 2. The MAR index of the isolates of ME 3 and ME 4 ranged between 0.2 to 0.5 and 0.14 to 0.41 respectively. Highest MAR index among all the ME ponds was observed in ME 1 pond for 8 isolates, followed by ME 2 with 0.6 MAR index (9 isolates). 100% incidence of MAR against more than 10 antibiotics was observed in ME 1 pond, followed by ME 2 and ME 3 with 88.6% and 23.4% resistance respectively. Multiple Antibiotic Resistance against 4–10 antibiotics was highest in ME 4 (90.5%) followed by ME 3 (76.7%) and ME 2 (11.4%) (Figure 4).

SI ponds

The pattern of multiple antibiotic resistance in vibrios from different ponds of SI farm is represented in figure 4. Out of 15 isolates from SI A ponds 20% of isolates exhibited resistance towards 10 antibiotics, 16.6% of SI B exhibited resistance towards 12 antibiotics. 25% isolates of SI C were resistant against 16 drugs and 10% isolates of SI D exhibited resistance towards 8 drugs.

In SI A pond, out of 15 isolates 9 isolates were resistant towards 4–10 antibiotics and one isolate against 3 antibiotics. 66.6% of isolates exhibited multiple antibiotic resistance. The MAR index ranged from 0.14 – 0.5. In SI B 58.3% exhibited multiple antibiotic resistance and MAR index varied from 0.04 to 0.73. Out of 12 isolates of SI B pond, 25% of isolates exhibited resistance towards more than 10 antibiotics and 33.4% to 4–10 drugs and 8.4% to 3 antibiotics.

In SI C, 66.7% isolates showed Multiple antibiotic resistance and MAR Index ranged between 0.04 and 0.72. 8.3% isolates were resistant to 3 antibiotics and 33.3% isolates were resistant to more than 10 and 4–10 antibiotics. 40% of isolates of SI D exhibited multiple antibiotic resistance and the MAR index ranged between 0.05–0.36. Out of 10 isolates 4 isolates were resistant towards 4–10 antibiotics and only one isolate is resistant to 3 antibiotics. No isolate was found to be resistant against more than 10 antibiotics (Figure 5). One way ANOVA test revealed that there is significant difference between multiple antibiotic resistance of ME and SI ponds ($p<0.05$).

V. harveyi, the causative agent of luminous vibriosis has been identified as the most pathogenic bacteria, responsible for mass mortalities in shrimp hatcheries (Lighter *et al.*, 1988; Lavilla Pitago *et al.*, 1990, 1992; Tonguthai, 1992; Karunasagar *et al.*, 1994) as well as in grow-out ponds (Lightner *et al.*, 1992; Abraham *et al.*, 1997; Jayasree *et al.*, 2007, 2008, 2012; Janakiram *et al.*, 2013). The luminous vibrios isolated from diseased shrimp are highly virulent with LC_{50} values as low as 1x 10^3 cfu / ml (Jayasree *et al.*, 2008) and exhibited multiple antibiotic resistance (Abraham *et al.*, 1997; Uma *et al.*, 2003; Manjusha *et al.*, 2006).

Sengupta *et al.* (2003) studied antibiotic sensitivity of 175 isolates of *Vibrio* from extensive, modified - extensive and semi-intensive shrimp culture ponds and observed high susceptibility of isolates to chloramphenicol (96%) and gentamycin (82%) and low susceptibility towards oxytetracycline. The present study on the resistance of *Vibrio* isolates from ME ponds and SI ponds, revealed that the isolates from SI ponds were more susceptible to antibiotics compared to the isolates of ME ponds. A similar observation was made by Abraham (2009). According to him increased resistance to OTC in shrimp farms of West Bengal was apparently due to the extensive use of this antibiotic for controlling vibriosis. Shotts *et al.*, (1976) reported that the bacterial pathogens easily develop plasmid mediated resistance to OTC and may also enhance the frequency of new OTC resistant bacteria in culture system (Abraham *et al.*, 1997). During the present study, most of the isolates of *V. harveyi* exhibited susceptibility towards OTC but were resistant to cephadroxil, clotrimazole. cloxacillin, co-trimoxazole, furazolidin, metronidazole, pefloxacin and penicillin - G. Isolates from all the ponds were sensitive to ciprofloxacin, erythromycin, norfloxacin and oxytetracycline but the degree of sensitivity varied from isolate to isolate. Uma *et al.* (2003) reported that *V. harveyi* isolated from shrimp affected with swollen gut syndrome were highly sensitive to the common antibiotics, chloramphenicol and gentamycin and resistant to Furazolidin. It is evident that no uniform pattern exists in the susceptibility / resistance pattern of isolates of *Vibrio* sp.

The present study revealed that the isolates from all the shrimp culture ponds (SI and ME) were resistant to cephadroxil, clotrimazole, co-trimoxazole, furazolidin, metronidazole, pefloxacin and penicillin – G, and the resistance varied from isolate to isolate, with maximum resistance towards penicilin G. Most of the isolates from ME 1 pond were resistant to more than three antibiotics and minimum resistance was observed in isolates from SI D pond. Manjusha *et al.* (2005) reported interesting results on antibiotic resistance of *Vibrio* sp. with highest antibiotic resistance to amoxycillin, ampicillin, penicillin, cefedoxime, rifampacin and streptomycin and lower resistance to chloramphenicol, tetracycline, chlortetracycline, furazolodine, nalidixic acid, gentamycin, sulphafurazle, trimethoprim, neomycin and amikacin which were commonly used in aquaculture farms through feeds during culture and hatchery production of seed. Seong Wei Lee *et al.* (2009) reported that the *Vibrio* spp. isolated from the marketable-sized white leg shrimp was found 100% sensitive to eight antibiotics (oxolinic acid, chloramphenicol, florfenicol, kanamycin, nalidixic acid, novobiocin, nitrofurantoin, and doxycycline), whereas *Vibrio* spp. isolated from the post larval white leg shrimp was found sensitive to only three antibiotics (kanamycin, nalidixic acid and florfenicol).

Saba Riaz *et al* (2011) mentioned that MAR is considered as a good tool for risk assessment and it gives an idea of the number of bacteria showing antibiotic resistance in the risk zone in the study's routine susceptibility testing. This MAR index also recommended that all isolates originated from the environment where antibiotics were over used (Paul *et al.*, 1997). Multiple antibiotic resistance of *Vibrio* species isolated from cultured shrimp has been reported earlier (Abraham *et al.*, 1997, 2009; Leano *et al.*, 1999; Eleonor and Leobert, 2001; Manjusha *et al.*, 2005).

Table.1 Percent isolates of *Vibrio harveyi* exhibiting Antibiotic Resistance towards different antibiotic drugs

S.No	Drug	ME 1	ME 2	ME 3	ME 4	SI A	SI B	SI C	SI D
1	Amoxicillin	100	62.8	26.7	47.6	0	25	41.7	0
2	Ampicillin	58.3	48.6	20	47.6	0	8.3	33.3	0
3	Cephadroxil	83.3	62.8	30	42.9	40	41.7	58.3	20
4	Cefazolin	100	74.3	73.3	71.4	13.3	25	50	0
5	Chloramphenicol	100	100	33.3	45.6	13.3	8.3	58.3	0
6	Ciprofloxacin	0	0	0	0	0	8.3	0	0
7	Clotrimazole	62.5	100	63.3	19.1	26.7	25	25	20
8	Cloxacillin	58.3	100	70	0	26.7	41.7	25	10
9	Co-Trimoxazole	79.2	48.6	36.7	37.6	26.7	66.7	41.7	20
10	Erythromycin	20.8	11.4	0	33.3	6.7	8.3	8.3	0
11	Furazolidin	37.5	60	53.3	61.9	40	33.5	25	20
12	Gentamycin	37.5	0	20	0	6.7	0	16.7	0
13	Metronidazole	100	100	100	80.9	40	50	58.3	50
14	Nitrofurazone	100	100	100	80.9	53.3	41.6	66.7	50
15	Norfloxacin	0	0	0	0	0	25	0	0
16	Oxytetracycline	50	54.3	0	0	6.6	0	8.3	0
17	Pefloxacin	100	37.2	23.3	28.6	26.7	25	41.7	10
18	Penicillin - G	100	100	100	100	100	100	100	100
19	Rifampacin	100	37.2	0	0	13.3	8.3	33.3	10
20	Streptomycin	70.8	28.5	0	0	6.7	0	25	0
21	Tetracycline	54.2	34.3	0	0	6.7	8.3	25	0
22	Trimethoprim	62.5	48.6	63.3	0	26.4	50	41.7	10

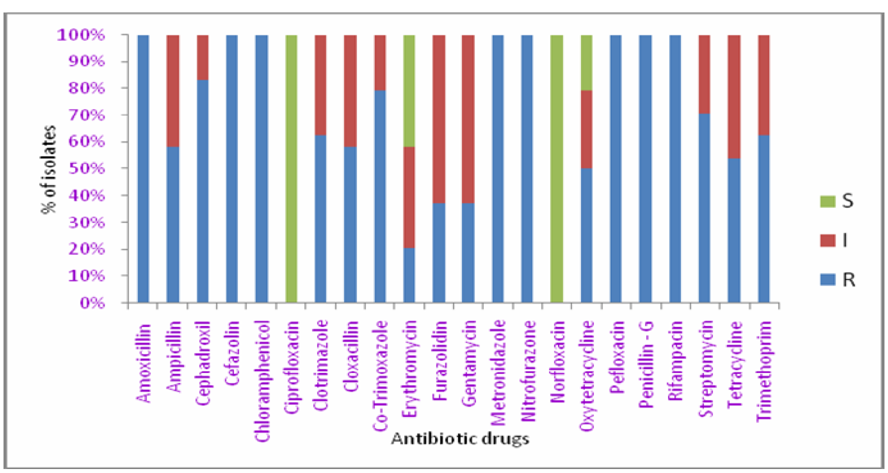
Table.2 Percent isolates of *Vibrio harveyi* exhibiting Antibiotic Susceptibility towards different antibiotic drugs

S.No	Drug	ME 1	ME 2	ME 3	ME 4	SI A	SI B	SI C	SI D
1	Amoxicillin	0	0	0	0	80	41.7	41.7	90
2	Ampicillin	0	0	0	0	73.3	50	41.7	100
3	Cephadroxil	0	0	0	0	33.3	25	33.3	50
4	Cefazolin	0	0	0	0	13.3	50	25	40
5	Chloramphenicol	0	0	46.7	9.5	73.4	50	33.4	80
6	Ciprofloxacin	100	100	100	100	66.7	50	100	80
7	Clotrimazole	0	0	0	0	20	25	25	10
8	Cloxacillin	0	0	0	33.3	33.3	25	41.7	50
9	Co-Trimoxazole	0	0	0	0	20	25	25	20
10	Erythromycin	41.7	37.2	66.7	66.7	73.3	75	75	80
11	Furazolidin	0	0	0	0	6.7	41.7	25	20
12	Gentamycin	0	11.4	43.3	66.7	46.7	66.7	66.6	70
13	Metronidazole	0	0	0	0	13.3	33.3	25	10
14	Nitrofurazone	0	0	0	0	13.3	25	25	20
15	Norfloxacin	100	100	100	100	60	75	91.7	90
16	Oxytetracycline	20.8	11.4	43.3	100	60	91.7	66.7	80
17	Pefloxacin	0	28.5	36.7	23.8	33.3	66.7	33.3	50
18	Penicillin - G	0	0	0	0	0	0	0	0
19	Rifampacin	0	0	43.3	38.1	60	41.7	58.4	80
20	Streptomycin	0	37.2	40	80.9	60	50	50	70
21	Tetracycline	0	40	50	100	60	58.4	58.4	70
22	Trimethoprim	0	0	0	47.6	26.7	25	50	40

Table.3 Percent isolates of *Vibrio harveyi* exhibiting Intermediate Antibiotic Susceptibility towards different antibiotic drugs

S.No	Drug	ME 1	ME 2	ME 3	ME 4	SI A	SI B	SI C	SI D
1	Amoxicillin	0	37.2	73.3	52.4	20	33.3	16.6	10
2	Ampicillin	41.7	51.4	80	52.4	26.7	41.7	25	0
3	Cephadroxil	16.7	37.2	70	57.1	26.7	33.3	8.4	30
4	Cefazolin	0	25.7	26.7	28.6	73.4	25	25	60
5	Chloramphenicol	0	0	20	42.8	13.3	41.7	8.3	20
6	Ciprofloxacin	0	0	0	0	13.3	41.7	0	20
7	Clotrimazole	37.5	0	36.7	80.9	53.3	50	50	70
8	Cloxacillin	41.7	0	30	66.7	40	33.3	33.3	40
9	Co-Trimoxazole	20.8	51.4	63.3	52.4	53.3	8.3	33.3	60
10	Erythromycin	37.5	51.4	33.3	0	20	16.7	16.7	20
11	Furazolidin	62.5	40	46.7	38.1	53.3	25	50	60
12	Gentamycin	62.5	88.6	36.7	33.3	46.7	33.3	16.7	30
13	Metronidazole	0	0	0	19.1	46.7	15.7	16.7	40
14	Nitrofurazone	0	0	0	19.1	33.3	33.3	8.3	30
15	Norfloxacin	0	0	0	0	40	0	8.3	10
16	Oxytetracycline	29.2	34.3	56.7	0	33.3	8.3	25	20
17	Pefloxacin	0	34.3	40	47.6	40	8.3	25	40
18	Penicillin - G	0	0	0	0	0	0	0	0
19	Rifampacin	0	62.8	56.7	61.9	26.7	50	8.3	10
20	Streptomycin	29.2	34.3	60	19.1	33.3	50	25	30
21	Tetracycline	45.8	25.7	50	0	33.3	33.3	16.6	30
22	Trimethoprim	37.5	51.4	36.7	52.4	46.6	25	8.3	50

Figure.1 Total percent susceptibility of *Vibrio harveyi* isolated from ME ponds, towards different antibiotics



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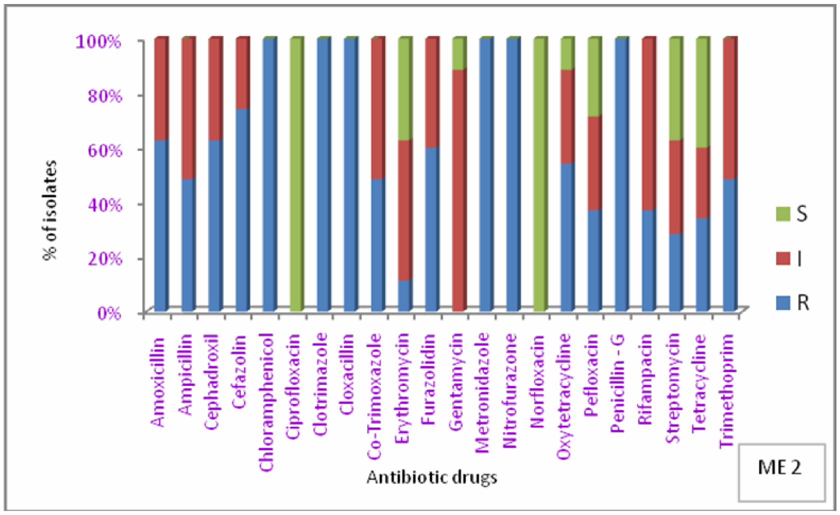
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Cloxacillin
Co-Trimoxazole
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Furazolidin
Gentamycin
Metronidazole
Nitrofurazone
Norfloxacin
Oxytetracycline
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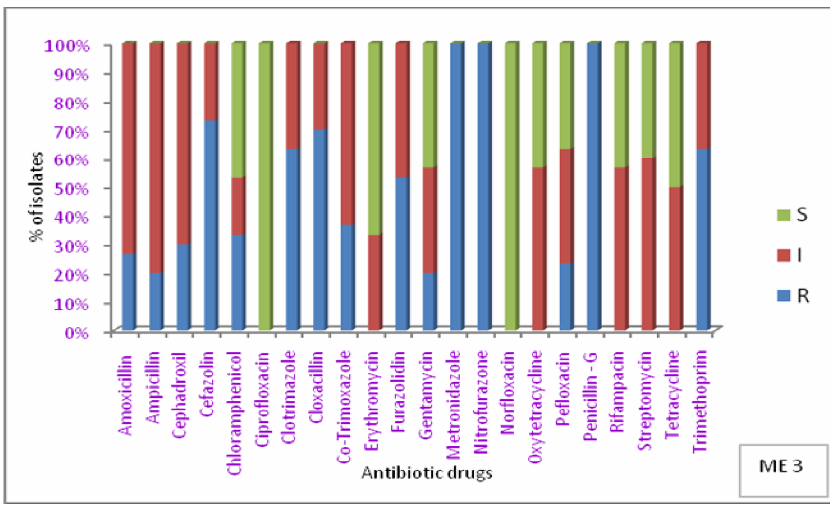
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Chloramphenicol
Ciprofloxacin
Clotrimazole
Cloxacillin
Co-Trimoxazole
Erythromycin
Furazolidin
Gentamycin
Metronidazole
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Norfloxacin
Oxytetracycline
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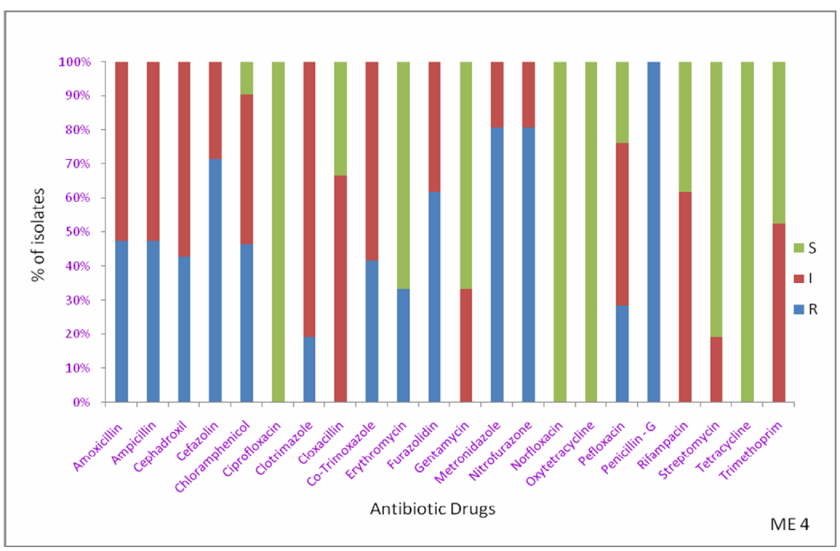
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Co-Trimoxazole
Erythromycin
Furazolidin
Gentamycin
Metronidazole
Nitrofurazone
Norfloxacin
Oxytetracycline
Pefloxacin
Penicillin - G
Rifampacin
Streptomycin
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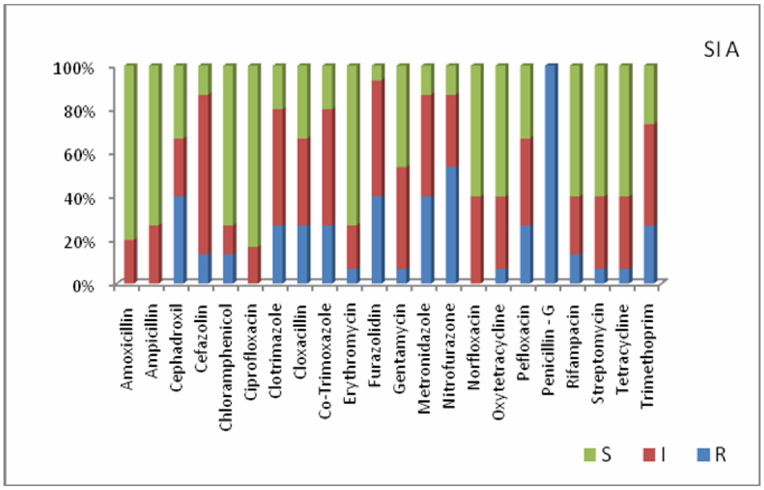
ME 4

Figure.2 Total percent susceptibility of *Vibrio harveyi* isolated from SI ponds, towards different antibiotics



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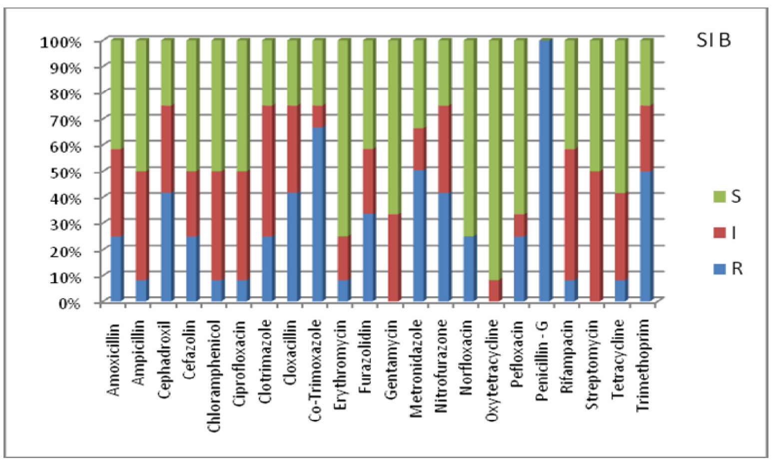

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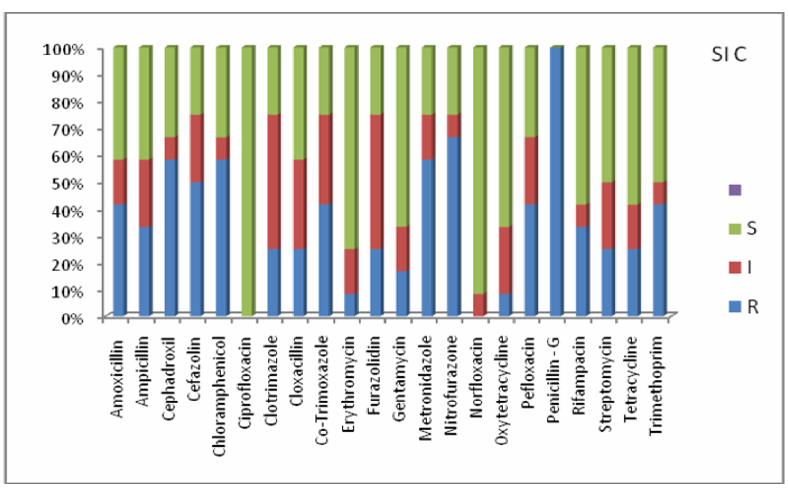

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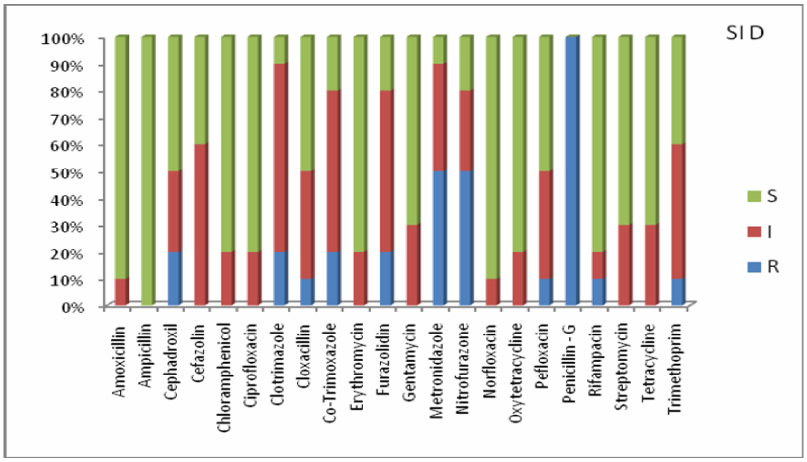
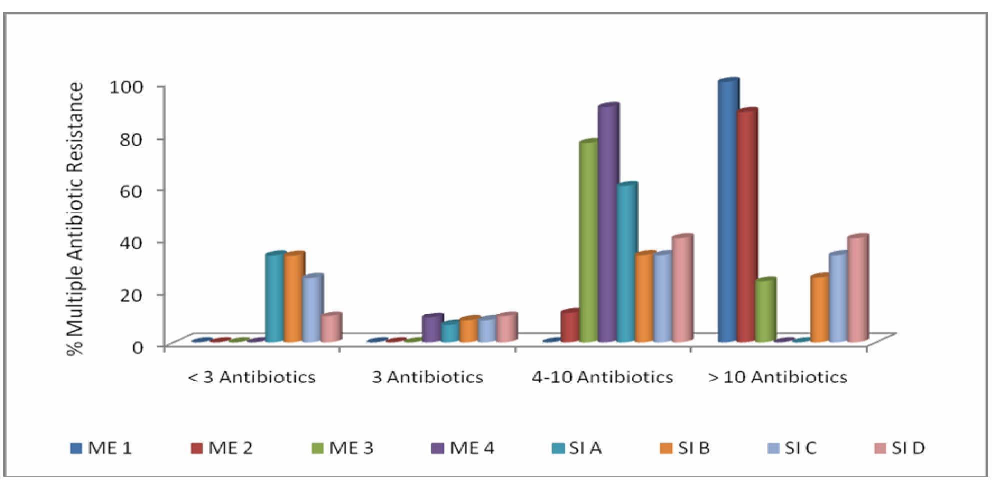


SI D

Figure.3 Multiple Antibiotic Resistance Index (MARI) of *Vibrio harveyi* isolated from shrimp culture (ME and SI) Ponds

Multiple Antibiotic Resistance Index (MARI)

Figure.4 Multiple Antibiotic Resistance (%) of *Vibrio harveyi* from ME and SI ponds



Abraham *et al.* (2009) reported that there was significant difference in the MAR of the isolates from Extensive pond (54.5%), (Semi-intensive) 33.3% and Modified Extensive (52.69%). They also mentioned that the MAR of *Vibrio* isolates revealed the acquired antibiotic resistance. In the present study, higher values of MAR index were observed in ME ponds, compared to those of SI ponds. The average MAR index values of *V. harveyi* isolated from different areas ranged between 0.31 to 0.67 in ME ponds and were 0.14 to 0.35 in SI ponds. The value of MAR index indicates the risk phase and if the value falls between 0.200 and 0.250, it becomes a very risky phase, where there are equal chances that MAR may fall in the high risk and low risk phases (Krumperman *et al.,* 1983). MAR index higher than 0.2 identifies organisms that originate from high risk sources of contamination, where antibiotics were often used (Freeman *et al.,* 1989; Orozova *et al.,* 2008). Seong Wei Lee *et al.,* (2009) reported that the study of MAR index indicated the postlarvae of white leg shrimp to be highly exposed to the tested antibiotics compared to marketable shrimp, which also implies that the marketable-sized white leg shrimp were safe for human consumption. From our studies, it is evident that ME ponds, where the isolates of *Vibrio* exhibited more resistance towards several antibiotics may be the result of continuous and indiscriminate usage of antibiotics in those ponds. The variation in the antibiotic resistance of isolates from different culture systems could be due to the differences in frequency in the usage of antibiotics, disease occurrence and management practices. Development of antibiotic resistance in shrimp farms and incidence of MAR are of great concern to shrimp aquaculture industry. Due to these antibiotics, it can be presumed that anthropogenic factors might have influenced in acquiring resistance by *Vibrio* spp., as there are no reports available on the use of these drugs for aquaculture in India (Manjusha *et al.,* 2005). In India, Marine Products Export Development Authority (MPEDA) has banned the usage of 19 antibiotics in Indian shrimp culture (MPEDA 2001). Therefore, the application of continuous and indiscriminate usage of antibiotics against shrimp diseases, both in culture ponds and hatcheries, should be avoided and preventive measures be taken to control disease problems by proper management practices.

Acknowledgements

The financial support provided by Department of Science and Technology, New Delhi (to Jayasree Loka) and University Grants Commission, New Delhi (to Janakiram Pasupuleti) to carryout this work is greatly acknowledged.

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