Detection of Virulence and Tetracycline Resistance Determinants in Aeromonas Spps. Isolated From Indian Ruho Carp (*Labeorohita*) Frys

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Abstract

An increasing incidence of multidrug resistance among Aeromonas spp. isolates, which are fish pathogens and emerging opportunistic human pathogens, has been observed in Indian ruho carp in India. This can be attributed to the horizontal transfer of genetic elements through plasmids. *(ompW, aha1, lip)* virulent genes (75%, 75%, 25%) were all present in (66.7%), (33.3%) *A. sobria*, *A. hydrophila* strains from thirty Indian ruho carp frys from aquaculture ponds respectively, while none of the isolates were bearing to *aero* virulence gene. One to seven resistant plasmids were isolated from all eight virulent and non virulent Aeromonas spp. showed multi-drug resistance with a molecular weight ranging from (1.1-23 kbp), the plasmid content of each isolate was examined using the alkaline lysis protocol. The antimicrobial susceptibilities of 8 Aeromonas spp. isolates from Indian Ruho carp (*Labeo rohita*) were determined by disc-diffusion testing. Gentamycin, Ciprfloxacin were the drug of choice in combating the bacterial growth in vitro (100%) sensitivity while high levels of resistance against ampicillin were observed (100%) while nalidixic acid and tetracycline showed some level of resistance (57.1%), (28.6%) respectively. tet determinant type was determined by amplification using six degenerate primer sets(tet*A, B, C, D, E, G*). Genomic and plasmid encoded *tetA*, *tetE* were observed while *tetG* was in coexistence in plasmid DNA only of the same isolate. Moreover *tetE* in genomic and plasmid born in the same another isolate were observed, However *tetB,C,D* were not detected in any of the isolates. The results indicate that the pond-raised Ruho carp may be a source of pathogenic Aeromonas spp. and that the

potential health risks posed by virulent and multiple antibiotic resistance strains of *Aeromonas* spp. should be estimated.
Keywords*:* Aeromonas, Virulent genes, Plasmid, Antibiotic resistance, *tet*.

Introduction

Labeo rohita, commonly called as Rohu and one of the three Indian major carps, is an important freshwater fish species normally cultured in Asia, particularly in the Indian region ***(Khan et al., 2004)***. Rohu carp culture related to about 35% of the total Indian major carp production ***(FAO, 2001)***. In India, incidence of *Aeromonas* spps is reported from various foods of animal origin via fish, seafood, raw and cooked meat, vegetables, milk and milk products ***(Agarwal et al., 2000),*** however, reports on detection of virulence and drug resistance genes are still limited ***(Kore et al., 2014)***. *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas veronii bv. sobria* contamination was detected in all or most of raw and ready to eat indian major carps, tilapia and shrimp in Kolkata, west bengal state, India ***(Manna et al., 2013)***. A wide range of putative virulence factors have been detected and studied in several Aeromonas spp**.** ***(Sechi et al., 2003)****;* Additionally, it has been shown that protein layers, O-antigens, fimbriae and outer membrane proteins of *A. hydrophila* play essential role of adherence of mechanism and contribute to colonization of fish tissue ***(Khushiramani et al., 2007)*** they play a pivotal role in the establishment of infection. There is an increasing incidence of antimicrobial resistance among Aeromonas spp. isolated from aquaculture environments ***(Schmidt et al., 2001a, b).*** Antimicrobial resistance genes used in aquaculture systems may be transmitted to human pathogenic bacteria ***(Smith et al., 1994)***. Plasmids containing multiple antimicrobial resistance determinants could potentially be transferred in natural microenvironments between bacterial pathogens of fish, humans suggesting the spread of mobile genetic elements such as plasmids from fish pathogens to human pathogens ***(Sørum, 1998)***.

A number of mobile genetic elements, including plasmids and transposons have been found in association with both clinical and environmental Aeromonas isolates ***(Schmidt et al., 2001a, b).*** Acquiring of new genetic material by susceptible bacteria from resistant strains often facilitates the coexistence of the multiple resistance genes into the host's genome or plasmids ***(Tenover, 2006),*** so the aim of the present study was to estimate the virulence of *Aeromonas* spp. in ponds of Indian ruho carp and to detect genomic and plasmid antimicrobial

resistance mediated by their specific genes.

2. Materials and methods

2.1. Isolation and identification of bacterial isolates

Presumptive Aeromonas spps. were isolated from appeared pathognomic lesion from muscle and kidney of Indian ruho carp frys, 10-15g wt (*Labeo rohita*). Bacterial colonies were grown in BHI broth and kept at 37ºC/24hrs after that inoculated in Brain Heart Infusion agar and MaCconkey agar with 100 ug/ml ampicillin and kept 30ºC/24 h then the grown isolates were inoculated on a BHI agar 30ºC/24 h for further purification and other identification tests; All isolates were subjected to the gram stain, oxidase, catalase, vogues proskauer, bile esculine hydrolysis, indole, oxidation- fermentation, sugar fermentation tests ***(Popff and Ve'ron, 1976)***

2.2. Antibiotic susceptibility testing

Antibiotic susceptibility to 11 antimicrobial agents was determined using (HiMedia, Mumbai) antibiotic disks, on Muller- Hinton agar (Oxoid) plates following CLSI, 2011, the tested organisms were flooded on surfaces of the Muller Hinton agar by a sterile cotton swap by immersion the bacterial suspension adjusted to an optical density of 0.5 McFarland standard units then the discs gently pressed using sterile foreceps, then the plates were incubated and the zone diameter and interpretation of the results were recorded according ***(Bio-Merieux, 1984).***

2.3. Molecular characterization of different virulent genes in Aeromonas spps. isolates using polymerase chain reaction analysis

Genomic DNA was extracted from 8 isolates following the protocol described by ***(Ausubel et al., 1995)*** by using 4 ml of BHI inoculated broth freshly prepared 37 ºC /24 h after that the genomic DNA was checked for purification and concentration using nano Drop Spectrophotometer (Thermo, USA).

2.4. Plasmid DNA analysis

Plasmid DNA was isolated manually from the same eight isolates that were virulent and also the resistant ones to at least one antibiotic. Isolates were grown overnight on Brain heart infusion broth in 37°C and then 3 ml picked off then following the alkaline lysis method described by ***(Sambrook et al., 1989)*** to check the presence of antibiotic resistance genes in plasmid then examined by agarose gel electrophoresis in 0.8% agarose gels, ethidium bromide staining then the gel run at 80 volts for 3o min and then subjected to UV illumination.

2.5. Characterization of virulence genes in Aeromonasspps. plasmid DNA.

The bacterial isolates were tested for (*ompW*), (*aha1*), (*aerO*), (*lip*) virulence genes as previously described by ***(Maiti et al. 2009), (Santos et al., 1999), (Cascon et al.,***

1996) using specific primers with their cycling conditions for each primer as listed in Table (1). using (BIO RAD thermal cycler).Fifteen microlitre PCR products were mixed with 3 µl loading dye and 6 µl molecular weight marker in a separate lane in each gel then the gel run at 80 volts for 3o min.

2.5. Characterization of tetracycline resistance genes (A, B, C, D, E, G) in Aeromonas spps. plasmid DNA

Identification of tetracycline resistance genes was performed using PCR amplification with the specific primers listed in Table (1). according to ***(Menggen et al., 2007)***. Assays were carried out in 30 µl reactions using master mix: (Genei, Bangalore, India). All PCRs were subjected to an Initial denaturation and final extension for 5 min at 95°C and 72°C respective, and the subsequent cycling conditions are listed in **Table (2).** 15 microliters were mixed with 3 µl loading dye and 6 µl molecular weight marker in a separate lane in each agarose gel 0.8%, ethidium bromide staining then the gel run at 130 volts for 3o min, and viewed by UV transillumination.

3. Results and Discussion

Multiple antibiotic resistance dissemination in aquatic environment which affect human health after consumption and direct contact of contaminated water or diseased fishes through their resistance plasmid which therefore important to investigate the use of antibiotics in aquaculture and the associated resistance to other relevant bacteria**.** *Aeromonas hydrophila, Aeromonas sobria* were the most prevalent in this study, phenotypic identification were recorded as gram-negative, positive oxidase, catalase, indole, citrate utilization. A pinky to dark red colour in vogues proskauer, methyl red tests, no blackening of the media in bile esculine hydrolysis in identification of *A. sobria*, acid from glucose. Most of the isolates gave acidic reaction at the bottoms and alkaline surface of the slant with gas and H_2S production or no while the remaining gave both acidic bottom and slant with gas, sugar fermentative as ***(Cipriano, 2001)*** findings**.**

Aeromonas induced serious epidemics of ulcerative disease of fish in South East Asia and other regions of the world have been reported before ***(Roberts et al., 1992).*** Among all the Indian major carps, *Cirrhinus mrigala* was the most affected one followed by *Labeo rohita* and *C. catla*,this reflects our significance use of Indian ruho carp fish and prevalence of virulence and antibiotic resistance genes in this study. *Aeromonas sobria*and *Aeromonas hydrophila* were isolated from kidney and muscle with high prevalence of *A. sobria*. *Aeromonas hydrophila* was not isolated from any of the 7 samples

obtained from moribund crab in a study done by ***(Nielsen et al., 2001)***, these results were not in coordenance with the present study results which showed that *A. hydrophila* was isolated from moribund carp 5/15 (33.3%) *Aeromonas hydrophila* from *Labeo rohita* ponds while10/15 (66.7%) were *A. sobria*.

The pathogenesis of *Aeromonas* infections is multifactorial, as a wide variety of virulence factors produced by aeromonads, including hemolysins, cytotonic and cytotoxic enterotoxins, proteases, lipases, leucocidins, endotoxin, adhesions, that act as disease cause in the host ***(Merino et al., 1995)***. *Aerolysine, Lipase, Outer membrane protein, Aeromonas adhesion* genes were the virulence assessement in the present study, the virulence factors enable the bacteria to colonize, gain entry, establish, replicate, and cause damage in host tissues and invade the host defense system and spread, eventually killing the host ***(Yu et al., 2005)***, However, not all species of aeromonads produce all the toxins ***(Chopra and Houston, 1999).*** *aerA* gene was wide spread in *A. veronii* biotype *sobria* and *A. hydrophila* but less so in *A. caviae* in the studies of ***(Seethalakshmi et al., 2008)***, aerolysin gene were high prevalence in different strains of *A. hydrophila* in comparable with *A. veronii* while *A. veronii biovar sobria* isolated from freshwater fish was also reported to lack the aerA gene ***(Gonzalez-Serrano et al., 2002)*** which were partially agreed with our results which showed that nothing of *Aeromonas hydrophila* or *Aeromonas sobria* were positive to Aerolysin gene isolated from diseased carp frys, in contrast ***(Nawaz et al., 2010)*** indicated that 96.0% of the *A. veronii* isolates from catfish harbored the *aer*A gene. Lipases and hydrolipases are considered important virulence factors in *Aeromonas* spp. because they alter the structure of the cytoplasmic membrane of the host which then appeared its pathogenicity, especially if the aerolysin gene is present, this was previously investigated by ***(Nawaz et al., 2010)*** also lipases are considered important for bacterial nutrition ***(Pemberton et al., 1997)*** the limit of lipase gene was low in this study about one isolate only in *Aeromonas hydrophila*, 1/8 (12.5%) isolated from ruho carp frys harboured lipase gene. 5/8 (62.5%) *were A. sobria* isolates shared both *ompW* and *aha1*virulence genes, 1/8 (12.5%) *A. hydrophila* isolate shared both *ompW* and *lip* virulence genes while only one 1/8 (12.5%) isolate *A. hydrophila* was *aha1*gene bearing, none of the isolates were bearing to *aero* virulence gene as shown in Fig. 1, 2.

Multiple antibiotic resistance (MAR) has been registered for *Aeromonas hydrophila* isolated from freshwater fish farms in association with a wide variety of drugs, commonly used as feed additives ***(Vivekanandhan et al.,***

2002). Antibiotic resistant bacteria present in an aquaculture may be transferred to humans through wound infections after the exposure to contaminated water or fish ***(Petersen and Dalsgaard, 2003).*** Most environmental investigations about antibiotic resistance have only included *Aeromonas* spp. ***(Huddleston et al., 2006).*** Ampicillin resistance was widespread in aeromonads, our study investigated that all the virulent strains of *A. hydrophila* and *A. sobria* showed multi antibiotic resistance, 100% resistance to ampicillin, these findings were in tune with ***(Igbinosa and okosh, 2012; Ngoci et al., 2012; Kore et al., 2014; Michelle, 2015)***. Strains of *Aeromonas* isolated from rivers ***(Gon˜i-Urrizaet al., 2000)*** showed 59% resistance against nalidixic acid which is in tune with our finding in *Aeromonas* spp. from Indian carp frys(57.1%). This study showed that gentamycin was the most effective against *Aeromonas* isolates carp ciprinids(100%) sensitivity, which is in clear contrast to earlier findings where resistance percentages up to 23.5% ***(Ansary et al., 1992)*** and 3.6 % of the *A. hydrophila* strains resistance to gentamycin ***(Tha. Thayumanavan et al., 2003)***. Resistance to chloramphenicol, nalidixic acid, streptomycin, sulfamethoxazole-trimethoprim and tetracycline has been observed among isolates of *A. hydrophila*in tilapia *(Oreochromismossambica)* ***(Son et al., 1997)***, these findings were in tune with ***(Dias et al., 2012)*** however, it is in contrary to our results that chloramphenicol, streptomycin, sulfamethoxazole-trimethoprim were highly effective drugs in vitro on *Aeromonas* strains**,** this finding was accepted by ***(Sarria-Guzma'n et al., 2013; Ye et al., 2013; Kore et al., 2014)***. Tetracycline resistant aeromonads were isolated in ***(Schmidt et al., 2001)*** study from rainbow trout as our finding where *A. sobria* showed resistance to tetracycline (28.6%) carp frys which indicated that a majority of the isolates were resistant to sulfadiazine/trimethoprim, this result were in tune with ***(Jun et al., 2010)*** but not like to our finding that reveals 100 % sensitivity from *Aeromonas* spps from carp frys. 3rd generations cephalosporines were highly effective against aeromonads isolated from carp frys (Cefotaxime,100%), these results were accepted in ***(Ngoci et al., 2012; Sarria-Guzma'n et al., 2013)*** previous studies. The present study investigated that ciprofloxacin, gentamycin were drug of choice when tested in vitro showed 100% sensitivity, these finding were highly accepted by many previous studies ***(Nogoci et al., 2012; Khairulet al., 2013; Kore et al., 2014)***. Multi drug resistance may be the result of the spread of resistance genes among the isolated bacteria, the main problem involving the use of antibiotics against

*Aeromonas*infections is the development of resistance by these bacteria ***(Mitchell and Plumb, 1980)***, generally related to the presence of plasmids ***(Ansaryet al., 1992).***

One to seven plasmids were extracted and used as template for amplification of resistance elements and all aeromonas isolates harboured plasmids from Indian carp frys ranging from 1.1 kb – 23 kb in size, as shown in **Fig.3.**, Table.4, these results were nearer to 21 kb plasmid size in ***(Ngoci et al., 2012)*** studies, but not like to ***(Jacobs and chenia, 2007), (Das et al., 2009)*** which isolated about more than one plasmid with a maximum size 64kb, this variation may be attributed to the diversity of plasmid molecular consitituent.

From the antibiotic sensitivity testing results, the tetracycline resistant strains were two isolates of *A. sobria* isolated from fish muscles. PCR was done to detect six types of tet resistant genes (*tet A, B, C, D, E, G*) according to ***(Menggenet al., 2007)*** using primers to detect the corresponding tetracycline resistance genes in 2 *Aeromonas sobria* isolates. The genetics of tetracycline resistance in aeromonads has been investigated previously ***(Schmidt et al., 2001)***, among various *tet* genes, five classes of genetically discernible tetracycline resistance determinants (*tet*A to *tet*E) have been described in *Aeromonas* spp. ***(Balassiano et al., 2007)***. Five classes designated as A through E have been described among aerobic enteric gram-negative bacteria of genetically distinguishable tetracycline resistance determinants, There have been reports that have showed that the most predominant tetracycline resistance genes in *Aeromonas* spp. were *tet*A and *tet*E ***(Nawaz et al., 2006)*** and that *tet*A was plasmid borne ***(Schmidt et al., 2001)***, ***(DePaola et al., 1988)*** reported that a majority of the tetracycline resistant *A. hydrophila* strains from catfish contained either *tet*A or *tet*E, ***(L'Abee-Lund and Sorum, 2001)*** showed that *tet*A was more predominant than *tet*E in *A. salmonicida* strains from fish these results are incoordenance with the present study results that showed the coexistence of *tet*A and *tet*E in the strains in of *A. sobria* genomic and plasmid DNA from indian carp frys while *tet*G was plasmid born only in one *A. sobria* isolate from muscle, see Fig.4. ***(Ndiand Barton, 2011)*** detected *tet*C more predominant than *tet*A in *Aeromonas* strains from rainbow trout farms in Australia, however *tet*B, *tet*D, *tet*E were not detected in any of the strains, these records were in contrast to the study results that *tet*C wasn't detected in any of our *Aeromonas* isolates while ***(Jacobs and chenia, 2007; Igbinosa and Okosh, 2012)*** studies were in corroboration with our results of absence of *tet*C. In last decades, it has been reported that bacteria associated with humans,

animals, fish and plants have many resistance determinants in common. *tet*A are already known to disseminate between aquatic and human bacteria ***(Adams et al., 1998).***

Conclusion

*The present study showed a high frequency of multi-virulent determinants and multiple drug resistance among *Aeromonas* spps. isolated from Indian ruho carp *(labeo rohita)* and suggested aquaculture as a reservoir of resistant bacteria which may affect other aquatic community and therefore transmit to human. Virulence and resistance were encoded by genes previously wide spread in other Aeromonads.

*Ciprofloxacin, gentamycin, 3rd generation cephalosporins were the drugs of choice against *Aeromonas spp.* while ampicillin, tetracycline, nalidixic acid were not preferable in compating Aeromonads.

Table 1. *Primer pairs and amplicon sizes used to detect virulent determinants (ompW, aha1, aero, lip).*

Gene	Primer name	Primer Sequence (5'-3')	Product length (bp)	Reference
ompW	*omp*WF1 *omp*WR	ATGAAAAAGATCCTTCCTCT TCAGAAGCGATAGCCGACAC	600	**Maitie*t* al., (2009)**
aha1	*aha*1-F1 *aha*1-R	ATGAAAAAGACAATTCTGGCT TTAGAAGTTGTATTGCAGGG	1120	**Maitie*t* al., (2013)**
aero	Ah-aerF Ah-aerR	GC(A/T)GA(A/G)CCC(A/G)TCTATCC(A/T)G TTTCTCCGGTAACAGGATTG	252	**Santos *et* al., 1999**
lip	*lip*-F *lip*-R	AACCTGGTTCCGCTCAAGCCGTTGTTGCT CGCCTCGGCCCAGCAGT	760	**Cascon *et* al., (1996)**

Table 2. *Primer pairs and related amplicon sizes of antibiotic Resistance Genes (tet A, B, C, D, E, G),* ***(Menggenet al., 2007)***

Antimicrobials Resistance genes Forward primer (59-39)	Oligonucleotide primer sequences	Forward primer (59-39)	Reverse primer (59-39)	Size (bp)
Tetracycline	***tetA***	TTGGCATTCTGCATTCACTC	GTATAGCTTGCCGGAAGTCG	494
	tetB	CAGTGCTGTTGTTGTCATTA	GCTTGGAATACTGAGTGTTAA	571
	tetC	CTTGAGAGCCTTCAACCCAG	ATGGTCGTCATCTACCTGCC	418
	tetD	GCAAACCATTACGGCATTCT	GATAAGCTGCGCGGTAAAAA	546
	tetE	TATTAACGGGCTGGCATTTC	AGCTGTCAGGTGGGTCAAAC	544
	tetG	GCTCGGTGGTATCTCTGCTC	CAAAGCCCCTTGCTTGTTAC	550

Table 3.*Temperature and time conditions of the primers sets during PCR*

Gene(s) name	Cycling conditions *						Number of cycle
	Denaturation		Annealing		Extension		
	Temp	Time	Temp	Time	Temp	Time	
ompW	95°C	1 min	52°C	1 min	72°C	1 min	30
aha1	95^0C	1 min	52^0C	1 min	72^0C	1 min	30
aero	94^0C	30 s	55^0C	30 s	72^0C	30 s	30
lip	94°C	1 min	62°C	1 min	72°C	1 min	30
tet	94°C	30 s	55^oC	30s	72^oC	30 s	35

Initial denaturation and final extension for 5 min at 95°C and 72°C respective.

Table.4. *Plasmids numbers and molecular weights for 8 isolates (Kb).*

Isolates code number	Type of isolate	Plasmid No.	Plasmid size (kb)
1	*A. sobria* (Ms)	1	9.416
2	*A. sobria* (K)	1	1.1
3	*A. sobria* (Ms)	1	1.1
4	*A. hydophila* (Ms)	1	1.1
5	*A. hydrophila* (K)	7	1.8, 2.1, 3.5, 3.8, 4.4, 8, 9
6	*A. sobria* (K)	3	1.8, 4.8, 12
7	*A. hydrophila* (Ms)	2	1.5, 1.8
8		7	1.1, 1.7, 3.5, 3.8, 8, 9, 23

(Ms): Muscle, (K): Kidney; all isolates were virulent except isolate no.6 was non virulent A. hydrophila isolated from kidney.

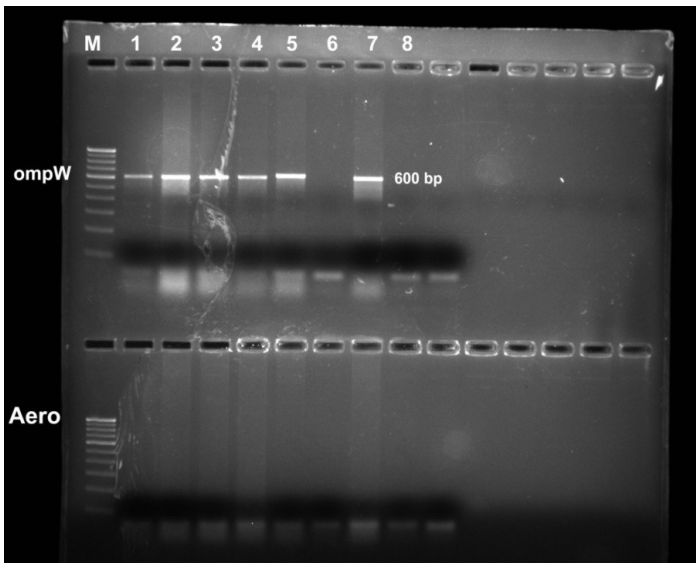

Fig. 1. *Agarose gel electrophoresis of PCR products of encodedompW and Aero. virulent genes from Aeromonas strains, lane M, Marker 100bp (Bangalore Genei™), lane 1,4 Aeromonas sobria taken from muscle, lane 2,3,7 Aeromonas sobria from kidney, lane 5 Aeromonas hydrophila from muscle, lane 6,8 non virulent Aeromonas hydrophila strain from kidney and muscle in both omp W and aer gene. The lower half of the gel shows negative results of aerolysin virulent gene of the same isolates in all lanes.*

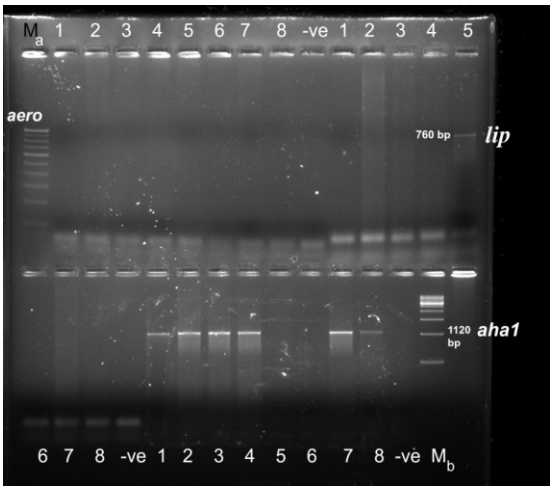

Fig.2. *Agarose gel electrophoresis of PCR products of encoded lip, aero, aha1.virulent genes from Aeromonas strains. lane Ma,100 bp Marker (Bangalore Genei*$^{TM),}$ *lane Mb, 500 bp Marker (Bangalore Genei*^{TM}*), negative results of aer gene at all eight isolates using Ma: 100 bp Marker (Bangalore Genei*^{TM} *), lane-ve, negative control, lane 5, is Aeromonas hydrophila positive lip gene giving 760bp positive band from muscle of Indian carp, lanes 2,3,7 are kidney isolates, 1 muscle isolate and all were biochemically identified as virulent Aeromonas sobria for aha1 virulent gene while isolate number 8 is a muscle isolate and was biochemically identified as a virulent Aeromonas hydrophila, lanes 5,6 were non virulent Aeromonas hydrophila taken from muscle and kidney respectively for aha1 virulent gene.*

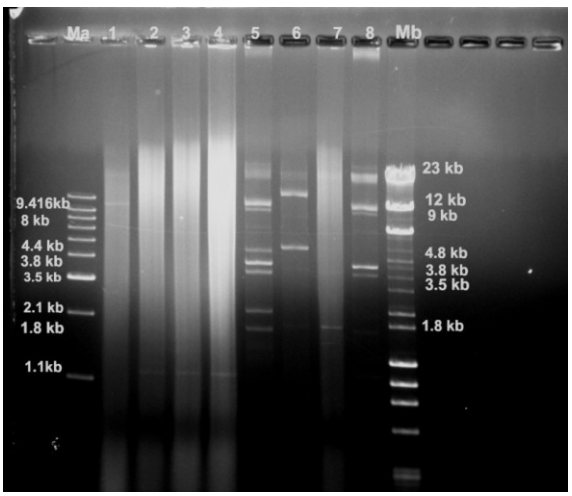

Fig.3. *Aeromonas spps. isolates bearing one to seven plasmids with sizes ranging from 1.1 kb – 23 kb, Ma: 1 kbp Marker (Bangalore Genei*^{TM} *), Mb: 23 kbp (l DNA - Hind III and fX174 DNA - HaeIII Mixas, finnzymes)*

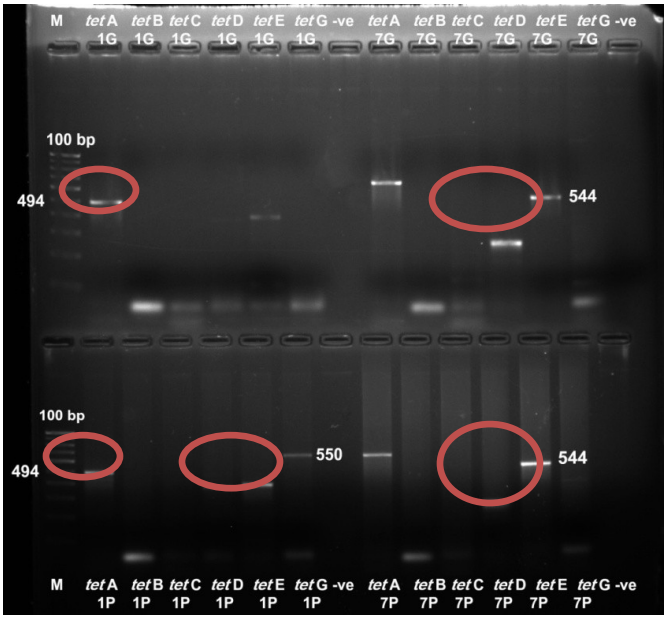

Fig.4. *PCR amplification of (tetA, B, C, D, E, G) genes from the template genomic and plasmid DNA of Aeromonas sobria, M : 100 bp Marker (Bangalore Genei™), 494 bp of tetA in genomic and plasmid from isolate 1(G,P), 550 bp of tetG in the plasmid of same isolate1P, 544 bp of tetE was present in genomic and plasmid DNA of the same isolate 7 (G, P).*

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الملخص العربي

بزيادة اكتشاف تعدد مقاومة بكتريا الايروموناس لمضادات البكتيرية المختلفة و التي تعتبر من البكتريا الممرضة للأسماك و من ثم غزو جسم الإنسان، تم عزل بكتريا الايروموناس من أسماك المبروك الهندي (الروهو الهندي), (*Labeo rohita*)، حيث يمنح نقل الجينات المقاومة للمضادات الحيوية عن طريق النقل الأفقي داخل جسم الكائن الحي عن طريق البلازميد، *(ompW, aha1,* (*lip* كجينات متخصصة في الضراوة تم ظهورها بنسبة (٧٥%، ٧٥%، ٢٥%) في بكتريا الايروموناس سوبريا و الايروموناس هيدروفيلا بنسبة تواجد ٦٦,٧% و ٣٣,٣% بالتابعية من ثلاثون سمكة من اسماك احواض تربية المبروك الهندي (الروهو)، و لم يتم اكتشاف جين الضراوة (*aerO*) في أياً من عزلات الدراسة. تم عزل واحد الي سبعة من البلازميد من ثمانية عينات حاملة لجينات الضراوة و غيرها غير ضارة و جميعهم مقاومين لواحد او اكثر من المضادات الحيوية بظهور حجم يتراوح من ١,١ الي ٢٣ كيلو بيز بير و تم استخدام طريقة التحليل القلوي في عزل البلازميد. تم تعرض الثمان عزلات الايروموناس علي المضادات الحيوية المختلفة و تبين ظهور الجينتاميسين و السيبروفلوكساسين بنسبة ١٠٠ % كدواء ناجح في مقاومة البكتريا علي العكس تم ظهور مقاومة عزلات البكتريا لعدة مضادات حيوية و هم الامبيسيللن و الناليديكسيك اسيد و التيتراسيكلين بنسبة ١٠٠% ، ٥٧,١%، ٢٨,٦% بالتابعية. تم تأكيد مقاومة العينات للتيتراسيكلين بالتعرض للمبادئ الخاصة بها من ٦ مجموعات (tet*A, B, C, D, E, G*) من عزلتان من الايروموناس سوبريا معزولان من عضلات اسماك الروهو الهندي و تم اكتشاف تواجد tet*A* في المحتوي الكروموسومي و بلازميد العزلة الاولي و tet*G*في محتوي البلازميد فقط من نفس العزلة ، كما تم التحقق من تواجد tet*E*في محتوي الكروموسومات و البلازميد في العزلة الثانية، كما اظهرت النتائج عدم ظهور كلا من (tet*B, C, D*) في عزلات الدراسة. نتائج الدراسة تشير الي ان احواض تربية سمك المبروك الهندي حاملة للميكروب الإيروموناس شديد الضراوة و المقاوم لعدة مضادات حيوية و من ثم يمثل خطورة علي بقية الاسماك المحيطة و بالتالي علي صحة الانسان.