Screening of Multi-Drug and Metal Resistant Aeromonas Species from Diverse Sources

Ramasamy Amsaveni, Muthusamy Sureshkumar, Joseph Reshma Mary, Umapathy Indra and Govindsami Vivekanandhan

Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore-641029, Tamilnadu, India
Farmer's Bio-Fertilizers and Organics 461, Sri Ragavendra Gardens Subramaniampalayam Road G.N. Mills Post Coimbatore-641 029, India

Abstract: The abuse of antibiotics in the modern era, lead the microorganisms to develop resistance. Antibiotic resistance becomes the part of natural selection in bacteria which allows them to survive in different environments. Bacteria like Aeromonas are able to adapt to changes in the environment such as an increase in antibiotic concentration, which often results in the development of mutations allowing them to survive in unfavourable conditions. The origin of antibiotic resistance in the environment is relevant to human health and there is an urgent need for predicting emerging resistant pathogenic microorganisms. As Aeromonas sp. has been reported as emerging pathogen, the multi-drug resistance was screened for the Aeromonas isolates obtained from fish intestine, clinical and environmental sources, against commercially available antibiotics and it was found that 95% of the isolates developed resistance towards at least one antibiotic. The emergence of antibiotic resistance in bacterial populations is a relevant field of study in molecular and evolutionary biology as well as in medical practice. The minimum inhibitory concentration of metals were performed for the isolates and it revealed that silver nitrate at 250 µM and copper sulphate at 8 mM concentration inhibited the growth of isolates. Further the metal resistance encoding genes, silP and copA were screened and it was found to be positive in 70% and 43% of the isolates, respectively.

Keywords: Aeromonas, Antibiotic Resistance, Metal Resistance

Introduction

Bacteria are among the most diverse living organisms and have adapted to a great variety of environments including the human body (Pizzaro-Cerda and Cossart, 2006). Aeromonas spp. are water-borne and food-borne, Gram-negative rods and oxidase positive bacteria. The 9th edition of “Bergey’s Manual of Determinative Bacteriology” classified Aeromonas into two main groups; the psychrophilic non motile and the mesophilic motile aeromonads (Parker and Shaw, 2010). Aeromonas have a broad host range and often been isolated from humans with diarrhoea (Ashdown and Koehler, 1993).

Sinha et al. (2004) in a study observed that the majority of Aeromonas strains exhibited a multidrug-resistance profile and this presents a significant threat to management of Aeromonas mediated diarrhoea. Multiple drug resistance among Aeromonas sp. has been reported from many parts of the world (Ko et al., 1996). Multiple Antibiotic Resistance (MAR) has been registered for A. hydrophila isolated from freshwater fish farms in association with a variety of drugs, commonly used as feed additives (Pettibone et al., 1996; Vivekanandhan et al., 2002). The universal and often indiscriminate use of antibiotics in human and animal medicine, including aquaculture, has brought serious consequences in the emergence of resistant strains of Aeromonas (Alvarez et al., 2004).

Levy (1992) stated that the over-reliance on the antibiotic agents made us to treat symptoms normally handled by our body's own immune system. The consequence to this reliance on antibiotic therapy was that bacteria developed ways to resist them. These strains reproduced and their offspring were also resistant, capable of causing infections not cured by antibiotic drugs. The increase in antimicrobial resistance poses a growing challenge in the treatment of Aeromonas infections (Lee et al., 2008).
Metals are directly or indirectly involved in all aspects of growth, metabolism and differentiation (Beveridge and Doyle, 1989). Heavy metal and antibiotic resistant competence of *A. hydrophila* have been studied by many researchers (Chandra and Monica, 2011; Odeyemi *et al.*, 2012). Heavy metals are stable and persistent environmental contaminants since they cannot be degraded or destroyed. Therefore, they tend to accumulate in soils and sediments (Montuelle *et al.*, 1994). In Tunisia, the persistence and proliferation of antibiotics and heavy metals resistance in bacterial pathogens, belonging to the *A. hydrophila*, in aquatic environments represents a considerable public health concern (Saïdi *et al.*, 2013). The structural and functional characteristics of antibiotic resistance share common themes with those of metal resistance (Baker-Austin *et al.* 2006). Woods *et al.* (2009) investigated the prevalence of silver resistance genes in 172 bacterial strains isolated from both human and equine wounds. They performed Polymerase Chain Reaction (PCR) screening for 8 genes, *silE, silRS, silP, silCBA* and *silF*.

**Materials and Methods**

**Sample Collection and Processing**

Fish specimens were randomly collected from fish retail outlets in sterile polyethylene bags and brought to the laboratory using an ice chest in less than an hour. The clinical diarrhoeal samples were collected from various hospitals in and around Coimbatore city, Tamil Nadu, India and transported to laboratory using Stuart’s transport medium. Subsurface soil samples were collected from mangrove region of Muthupetai, Tamil Nadu, India in sterilized plastic bags and transported to the laboratory. The samples were enriched and streaked on starch ampicillin agar medium (SAA) and incubated for 24 h at 37°C. A characteristic yellow to honey colored colonies were selected and used for further testing.

**Isolation of Presumptive Aeromonas Isolates**

After enrichment and streaking onto SAA, honey colored colonies were selected for enzymatic tests which includes oxidase and catalase. The oxidase and catalase positive colonies were then purified by repeated streaking on the nutrient agar and were maintained in the nutrient agar slants.

**Genotypic Identification of Aeromonads**

PCR was done for screening of the 16S rRNA and *rnpB* housekeeping genes by using genus specific primers with the expected amplicon size of 1050 bp and 410 bp respectively. The primer sequence was tabulated in Table 1. PCR of 10 µL reaction was performed with one isolate to optimize the conditions and a 15 µL reaction was performed for all the isolates. The PCR conditions followed are presented in Table 2. Each reaction was carried out using 7 µL of PCR master mix (Ampliqon, Denmark), 3 µL of nuclease free water, 1.5 µL of each forward and reverse primers (10pM) and 2µL of template DNA (50ng).

**Antibiotic Sensitivity Test**

Antibiotic sensitivity was tested for all the isolates used in this study. The antibiotics that are commonly used for the infection control such as Amoxycyclav (30mcg), Aztreonam (30mcg), Cephodoxime (10mcg), Cephalothin (30mcg), Chloramphenicol (30mcg), Gentamicin (10mcg), Rifampicin (5mcg), Streptomycin (25mcg), Tetracycline (30mcg), Vancomycin (10mcg) were selected for the present study. Antibiotic sensitivity test was carried out by disc diffusion method (Jorgensen and Ferraro, 2009). Muller Hinton agar (HiMedia, India) was prepared, sterilized and poured onto sterile petriplates. Pure cultures grown in nutrient broth were swabbed on the MHA plates and using antibiotic disc dispenser, discs were placed on the agar surface. After the incubation at 37°C for 18-24 h, the diameter of the inhibition zone was measured and compared with the interpretative chart provided by the manufacturer.

**Minimum Inhibitory Concentration (MIC)**

**MIC for Silver Nitrate (AgNO₃. 6H2O)**

MIC for silver nitrate was tested with Luria-Bertani (LB) agar. A wide range of silver nitrate concentrations were used to find out the MIC values. LB agar with silver nitrate concentrations like 0 µM, 10 µM, 30 µM, 50 µM, 100 µM, 150 µM, 200 µM and 250 µM were prepared by adding appropriate volume of silver nitrate from the stock. Overnight culture was diluted up to 10⁻⁸ bacterial cells/mL in sterile PBS solution. Dilutions of 10⁻⁶, 10⁻⁷ and 10⁻⁸ were inoculated as spots (5 µL) on the surface of plates using a micropipette. Plates were incubated at 37 °C for 24 h and were observed for growth.

**MIC for Copper Sulphate (CuSO₄.5H2O)**

LB agar with copper sulphate concentrations like 0 mM, 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM and 7 mM were prepared. It was allowed to cool to 45-50 °C and appropriate volume of copper sulphate from stock was added to the medium to get the required concentration. The content of the flask was mixed well and poured into sterile petriplates. The overnight culture was diluted to 10-8 bacterial cells/mL in sterile PBS solution. Dilutions of 10⁻⁶, 10⁻⁷ and 10⁻⁸ were inoculated as spots (5 µL) on the plates with copper sulphate using micropipette. Plates were incubated at 37°C for 24 h and were observed for bacterial growth.
Table 1. Primers used in this study

| S. No. | Genes | Primer sequence | Base pairs |
|--------|-------|-----------------|------------|
| 1      | 16S rRNA | F-5′ CAGAAGAAGCACCGGCTAAC 3′ R-5′ TTACCTTATTACGACTTCA 3′ | 1050 |
| 2      | rnpB   | F-5′ TGGGCAATCGCTGCTTCGT 3′ R-5′ AGGTCGGAGTCGGCCTGTAA 3′ | 400 |
| 3      | silP   | F-5′ AGTGCAACACAACAC 3′ R-5′ ACTTTCTCTGCACGGA 3′ | 1200 |
| 4      | copA   | F-5′ CTTTACGGACTTTTACCCGCC 3′ R-5′ GCCGCGGCCGCTTTGGGAGTTGAAAAC 3′ | 1300 |

Table 2. PCR conditions for the amplification of genes

| S. No. | Genes amplified | Initial denaturation (°C) | Denaturation (°C) | Annealing (°C) | Extension (°C) | Final extension (°C) | No. of cycles |
|--------|-----------------|---------------------------|-------------------|---------------|---------------|---------------------|--------------|
| 1      | 16sr RNA        | 95°/5min                  | 94°/30sec         | 52°/30sec | 72°/1min | 72°/5min | 30 |
| 2      | rnpB            | 95°/5min                  | 94°/30sec         | 54.5°/30sec | 72°/1min | 75°/5min | 30 |
| 3      | silP            | 96°/4min                  | 96°/20sec         | 54.2°/20sec | 72°/2min | 72°/5min | 35 |
| 4      | copA            | 95°/5min                  | 94°/1min          | 50.7°/40sec | 72°/1min | 72°/10min | 35 |

Detection of Metal Resistance Gene

PCR amplification of silP and copA was performed by using specific primers. The primers used in this study and reaction conditions were tabulated in Table 1 and Table 2.

Results

Incidence of Aeromonas from Various Sources

The incidence of Aeromonas spp. was recorded in the intestine of marine fish, soil and clinical (diarrhoeal) sources. A total of 130 samples were processed and 88 samples i.e., 68% showed positive for Aeromonas. Of the positive Aeromonas isolates, 79% were from fish intestine, 53% from soil and 31% isolates were of clinical origin (Table 3 and Fig. 1).

Antibiotic Sensitivity Assay

Multidrug resistance was shown by most of the isolates. Maximum level of resistance was shown to cephalothin by most of the isolates and least percentage of resistance was shown towards aztreonam. All the isolates were sensitive to chloramphenicol and gentamicin. Aeromonas isolates obtained from fish, of about 68%, 61%, 76% and 75% were found to be resistant to amoxyclav, cefpodoxime, cephalothin and vancomycin, respectively. Soil isolates of about 94%, 78%, 83% and 83% were found to be resistant to amoxyclav, cefpodoxime, cephalothin and vancomycin, respectively. About 100%, 82%, 91% and 100% of clinical isolates were found to be resistant to amoxyclav, cefpodoxime, cephalothin and vancomycin, respectively. With regard to amoxyclav, cefpodoxime, cephalothin and vancomycin, higher frequency of antibiotic resistance was recorded among the clinical isolates.

Resistance Pattern

The isolates exhibited 29 different resistance patterns. Among the total isolates, 95% showed resistance to atleast one antibiotic. Two of the isolates obtained from fish showed resistance towards a single antibiotic. The F55 isolate showed resistance towards a maximum of seven antibiotics. Four of the isolates (F56, S70, C83 and C84) were found to be resistant to six antibiotics with 3 different resistance patterns. About 17% of the isolates developed resistance against five antibiotics with 5 patterns of resistance. Further 33% of the isolates showed resistance to about 4 antibiotics with 9 different patterns for the antibiotics used. The multiple antibiotic resistance is prominent among fish isolates when compared with the soil and clinical isolates.

MAR Index of the Isolates

The MAR index value of all the isolates ranged from 0.2 to 0.7. MAR index of 0.2 to 0.3 was exhibited by 40% of the isolates. MAR index of 0.4 to 0.5 and 0.6 to 0.7 was exhibited by 54% and 6% of the isolates, respectively. The MAR index in the range of 0.2 to 0.3 was shown by 41%, 28% and 37% of the fish, soil and clinical isolates, respectively. About 46%, 67% and 45% of the respective fish, soil and clinical isolates showed the MAR index ranging from 0.4 to 0.5. Very few isolates showed the MAR index ranging from 0.6 to 0.7, which includes 3%, 6% and 18% of the fish, soil and clinical isolates, respectively. About 10% of the sensitive isolates were recorded among fish isolates. None of the soil and clinical isolates exhibited MAR index <0.2.
Table 3. The percentage of incidence of *Aeromonas* spp.

| Samples | No. of samples | No. of samples positive | Percentage of incidence |
|---------|----------------|-------------------------|-------------------------|
| Fish    | 75             | 59                      | 79                      |
| Soil    | 34             | 18                      | 53                      |
| Clinical| 35             | 11                      | 31                      |
| Total   | 130            | 88                      | 68                      |

Fig. 1. Lane M- Marker 100-1000 bp. 1a-amplification of 16S rRNA gene, amplicon-1050bp; 1b-amplification of rnpB gene, amplicon-400bp; Note: Distinct bands indicate the amplification of 16S rRNA and rnpB genes of *Aeromonas* isolates

**Minimum Inhibitory Concentration of Metals**

**MIC of Silver Nitrate**

The inhibition of bacterial growth by silver nitrate was observed for different concentrations such as 0 µM, 10 µM, 30 µM, 50 µM, 100 µM, 150 µM, 200 µM and 250 µM. The LB agar without silver nitrate was used as a control. At 250 µM concentration, none of the isolates exhibited the resistance to silver nitrate. About 14% showed resistance to 200 µM concentration of silver nitrate, which comprises 11%, 18% and 15% of isolates isolated from fish, soil and clinical sources, respectively. At 150 µM concentration of silver nitrate, 49% of isolates showed resistance among which, 52%, 50% and 33% of fish, soil and clinical isolates, respectively. Whereas, at 100 µM and 50 µM concentrations of silver nitrate, 92% and 98% of resistant isolates were observed. However, the minimal concentrations of silver nitrate (10 µM and 30 µM) do not interfere with the growth of the isolates.

**MIC of Copper Sulphate**

Inhibition of bacterial growth was observed on LB agar medium supplemented with 0 mM to 7 mM of copper sulphate concentrations. None of the test isolates showed resistance to 7 mM concentration of copper sulphate. At the concentration of 6 mM, 25% of the isolates showed resistance to copper sulphate, among which 26%, 23% and 21% from fish, soil and clinical isolates, respectively. About 63% of the isolates (63% of fish and soil isolates and 70% of clinical isolates) showed resistance to 5 mM concentration of copper sulphate, whereas at 4 mM concentration of copper sulphate, 97% of the isolates exhibited growth. As the lower concentrations of copper sulphate (1 mM, 2 mM and 3 mM) do not have much effect, hence all the isolates exhibited the growth on LB agar plate.

**silP Gene**

The gene coding for silver resistance (*silP*) was screened in all the isolates which is of 1200 bp. About 62 isolates (70%) were found to be conserved with this gene. Of which, about 81%, 61% and 27% of isolates from fish, soil and clinical isolates, respectively were conserved with *silP* gene. The *silP* gene was highly conserved among the fish isolates when compared to soil and clinical isolates. The presence of *silP* gene do not have complete role in existence of silver resistance among the isolates screened for varying concentrations of silver nitrate except for some isolates. The presence of resistance gene and the development of silver resistance were not in correlation in all the isolates. Few of the
isolates possess the silP gene but it could not resist the higher concentrations of silver and fewer shows the resistance even at higher concentrations of silver without the presence of silP gene, which indicates that some other components in silver resistance gene cluster may plays vital role in development of resistance towards silver.

copa Gene

The presence of copper resistance (copA) was screened in all the isolates used in this study. Of the 88 isolates screened for copA gene coding for copper resistance (1300 bp), 38 isolates were found to be conserved with this gene. The copA gene was highly conserved among the fish isolates when compared with soil and clinical isolates since, among 43% existence, 51%, 17% and 45% was conserved in fish, soil and clinical isolates, respectively. As like the case of silver resistance, the presence of copA gene do not have complete role in existence of copper resistance among the isolates screened for varying concentrations of copper sulphate except for some isolates. So the complete cluster of copper resistance genes should be studied for determining the exact component which favours the microbes to resist copper.

Discussion

Incidence of Aeromonas Isolated from Various Sources

In the present investigation the prevalence of Aeromonas spp. was recorded in marine fish intestine, soil and clinical (diarrhoeal) sources. A total of 130 samples were processed and about 68% showed positive for Aeromonas. Higher prevalence of Aeromonas spp. was observed in fish samples when compared to other samples used in this current research, which indicates the opportunistic nature of Aeromonas spp. and it was also a normal flora of fish intestine. It was reported that fish may also be a vehicle for pathogenic bacteria naturally occurring in aquatic environments referred to as indigenous or derived from polluted waters and or from post capture contamination, storage and handling. Joseph et al. (2013) screened for the occurrence of Aeromonas spp. in tropical seafood, aquafarms and mangroves of Cochin coast in South India and they recovered 11% of Aeromonas spp. by 16S rDNA sequence analysis. In the present study about 53% of Aeromonas spp. was recorded in soil samples, which indicates aeronomads are ubiquitous in occurrence. The variability in the prevalence among West coast and east coast regions of South India may be due to many reasons like river flow and anthropogenic activities.

In the current research 31% of the diarrhoeal samples were found to be contaminated with aeronomads. The prevalence of Aeromonas spp. among clinical isolates were found to less when compared with other sources. This was supported by Oberhelman and Taylor (2000), who reported that the isolation rate of Aeromonas in many developing countries may range from 5 to 28% in clinical isolates. Similarly, the occurrence of A. hydrophila in acute gastroenteritis among children was reported in the Coimbatore region, Tamil Nadu by Subashkumar et al. (2004), where the clinical isolates were collected in the present study. Of the 216 samples they collected, 10% was positive for A. hydrophila.

Antibiotic Sensitivity

In recent years development of resistant or multidrug resistant pathogens has become a major problem in India and many countries (WHO, 2013). Bacterial resistance is closely associated with the use of antimicrobial agents in clinical practice. The aeronomads have been regarded as universally resistant to penicillins (penicillin, ampicillin, carbencillin and ticarcillin) for quite a long time. In the present study the utmost resistance was found to be developed against cephalothin by the isolates and least resistance was shown towards aztreonam. All the isolates were found to be sensitive to chloramphenicol and gentamicin. The F55 isolate obtained from fish showed resistance towards a maximum of seven antibiotics. Multidrug resistance was shown by most of the isolates. With regard to amoxyclyl, cefpodoxime, cephalothin and vancomycin, higher frequency of antibiotic resistance was recorded among the clinical isolates. All the isolates were found to be sensitive to chloramphenicol and gentamicin.

Minimum Inhibitory Concentration of Metals

Although some heavy metals are important and essential trace elements, at high concentrations most of them can be toxic to microbes. Silver et al. (1989) reported that most of the bacteria developed resistance mechanisms in order to survive the high concentrations of metals in the environment. Miranda and Castillo (1998) isolated antibiotic and metal resistant Aeromonas isolates from polluted and unpolluted waters.

In the present study, the minimum inhibitory concentration of silver nitrate towards the Aeromonas isolates used in this study is 250 µM. Among the isolates screened the clinical isolates have shown constant resistance till 150 µM, which indicates the prevalence of high silver resistance isolates from clinical source. The minimum inhibitory concentration of copper sulphate towards the Aeromonas isolates used in this study is 7 mM. From the results it is evident that high copper resistant isolates were predominant among the clinical and fish isolates. Several of the silver-resistant strains have been collected from silver-treated patients at burn centres, where these strains have sometimes caused outbreaks (Pirnay et al., 2003). Pike et al. (2002) screened for silver resistance on MHA containing 50 µM silver nitrate.
µM, 200 µM, 300 µM and 500 µM AgNO3 and their results correlate with the results of present study.

In the present investigation further silver (siP) and copper (copA) resistance genes which are of 1200 bp and 1300 bp, respectively were screened in all the isolates. The siP gene was conserved in 70% isolates and copA gene in 43% of the isolates. In both the cases of siP and copA resistance genes, higher prevalence was noticed in fish isolates when compared with the other isolates.

Conclusion

The genus Aeromonas is one of several medically important genus that have become an increasingly troublesome group due to its pathogenesis in human and in aquatic life. The emergence of antibiotic resistance in bacterial populations due to abuse of antibiotics is a major problem in the current situation. In the present study metal resistance of Aeromonas isolates were recorded from diverse sources. This study will be helpful to distinguish the virulent strains from normal Aeromonas flora.

Acknowledgment

Authors are thankful to Secretary, Principal and Department of Biotechnology of Kongunadu Arts and Science College for providing facilities and encouragement to carry out this research work.

Conflict of Interest

The authors declare that they have no conflict of interests.

Author’s Contribution

Ramasamy Amsaveni: Participated in all experiments, coordinated the data-analysis and contributed to the writing of the manuscript.

Muthusamy Sureshkumar: Participated in all experiments, coordinated the data-analysis and contributed to the writing of the manuscript.

Joseph Reshma Mary: Participated in performing molecular experiments.

Umapathy Indra: Participated in writing this research paper.

Govindasami Vivekanandhan: Designed the research plan and organized the study.

Ethical Statement

The authors declare that the manuscript has not been submitted to more than one journal for simultaneous consideration, has not been published previously, none of the data have been fabricated or manipulated.

References

Alvarez, J.D., C.P. Agurto, A.M. Alvarez and J. Obregon, 2004. Resistencia antimicrobiana en bacterias aisladas de tilapias, agua y sedimento en Venezuela. Revista Cientifica.

Ashdown, L.R. and J.M. Koehler, 1993. The spectrum of Aeromonas-associated diarrhea in tropical Queensland, Australia. Southeast Asian J. Trop. Med. Public Heal, 24: 347-353. PMID: 8266241

Baker-Austin, C., M.S. Wright, R. Stepanauskas and J.V. McArthur, 2006. Coselection of antibiotic and metal resistance. Trends Microbiol., 14: 176-182. DOI: 10.1016/j.tim.2006.02.006

Beveridge, T.J. and R.J. Doyle, 1989. Metal Ions and Bacteria. 1st Edn., Wiley, New York, ISBN-10: 0471629189, pp: 461.

Chandra, R. and S. Monica, 2011. Influence of lignin, pentachlorophenol and heavy metal on antibiotic resistance of pathogenic bacteria isolated from pulp paper mill effluent contaminated river water. J. Environ. Biol., 32: 739-745. PMID: 22471210

Joseph, A.V., R.S. Sasidharan, H.P. Nair and S.G. Bhat, 2013. Occurrence of potential pathogenic Aeromonas species in tropical seafood, aquafarms and mangroves off Cochin coast in South India. Vet. World, 6: 300-306. DOI: 10.5455/vetworld.2013.300-306

Ko, W.C., K.W. Yu, C.Y. Liu, C.T. Huang and H.S. Leu et al., 1996. Increasing antibiotic resistance in clinical isolates of Aeromonas strains in Taiwan. Antimicrob Agents Chemother, 40: 1260-1262. PMID: 8723478

Lee, M.F., C.F. Peng, Y.H. Lin, S.R. Lin and Y.H. Chen, 2008. Molecular diversity of class Iintegrons in human isolates of Aeromonas spp. from southern Taiwan. Jpn. J. Infec. Dis., 61: 343-349. PMID: 18806339

Levy, S.B., 1992. The Antibiotic Paradox. 1st Edn., Plenum Press, New York, pp: 279.

Miranda, C.D. and G. Castillo, 1998. Resistance to antibiotic and heavy metals of motile aeromonads from chilean freshwater. Sci. Total Environ., 224: 167-176. DOI: 10.1016/S0048-9697(98)00354-4

Montuelle, B., X. Latour, B. Volat and A.M. Gounot, 1994. Toxicity of heavy metals to bacteria in sediments. Bull Environ. Contam Toxicol., 53: 753-758. DOI: 10.1007/BF00196950

Oberhelman, R.A. and D.N. Taylor, 2000. Campylobacter infections in developing countries. Washington. Am. Soc. Microbiol., 2: 139-153.

Odeyemi, O.A., A. Asmat and G. Usup, 2012. Antibiotics resistance and putative virulence factors of Aeromonas hydrophila isolated from estuary. J. Microbiol. Biotechnol. Food Sci., 1: 1339-1357.
Parker, J.L. and J.G. Shaw, 2010. *Aeromonas* spp. Clinical microbiology and disease. *J. Infect.*, 62: 109-118. DOI: 10.1016/j.jinf.2010.12.003

Petitbone, G.W., J.P. Mea and B.M. Sampsell, 1996. Incidence of antibiotic and metal resistance and plasmid carriage in *Aeromonas* isolated from brown null head (*Ictalurus nebulosus*). *Lett. Appl. Microbiol.*, 23: 234-240. DOI: 10.1111/j.1472-765X.1996.tb00073.x

Pettibone, G.W., J.P. Mear and B.M. Sampsell, 1996. Incidence of antibiotic and metal resistance and plasmid carriage in *Aeromonas* isolated from brown null head (*Ictalurus nebulosus*). *Lett. Appl. Microbiol.*, 23: 234-240. DOI: 10.1111/j.1472-765X.1996.tb00073.x

Pike, R., P. Stapleton, V. Lucas, G. Roberts and R. Rowbury et al., 2002. Effect of medium composition on the susceptibility of oral streptococci to mercuric chloride. *Curr. Microbiol.*, 45: 272-276. DOI: 10.1007/s00284-002-3754-1

Pirnay, J.P., D. De Vos, C. Cochez, F. Bilocq and J. Pirson et al., 2003. Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: Persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. *J. Clin. Microbiol.*, 41: 1192-1202. DOI: 10.1128/JCM.41.3.1192-1202.2003

Pizzaro-Cerda, J. and P. Cossart, 2006. Bacterial adhesion and entry into host cells. *Cell.*, 124: 715-727. DOI: 10.1016/j.cell.2006.02.012

Saidi, N., R. Lagha, F.B. Abdallah, K.B. Rokbani and A. Bakhrouf, 2013. Slime producing, heavy metals and antibiotics resistance in *Aeromonas hydrophila* isolated in Tunisia. *Afr. J. Microbiol. Res.*, 7: 5697-5708.

Silver, S., T.K. Misra and R.A. Laddaga, 1989. Bacterial Resistance to Toxic Heavy Metals. In: *Metal Ions and Bacteria*, Beveridge T.J. and R.J. Doyle(Eds.), Wiley, New York, ISBN-10: 0471629189, pp: 461.

Sinha, S., T. Shimada, T. Ramamurthy, S.K. Bhattacharya and S. Yamasaki et al., 2004. Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata. *Indian J. Med. Microbiol.*, 53: 527-534. DOI: 10.1099/jmm.0.05269-0

Subashkumar, R., T. Thayumanavan, G. Vivekanandhan and P. Lakshmanaperumalsamy, 2006. Occurrence of *Aeromonas hydrophila* in acute gastroenteritis among children. *Indian J. Med. Res.*, 123: 61-66. PMID: 16567870

Vivekanandhan, G., K. Savithamani, A.A.M. Hatha and P. Lakshmanaperumalsamy, 2002. Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. *Int. J. Food Microbiol.*, 76: 165-168. DOI: 10.1016/S0168-1605(02)00009-0

Woods, E.J., C.A. Cochrane and S.L. Percival, 2009. Prevalence of silver resistance genes in bacteria isolated from human and horse wounds. *Vet. Microbiol.*, 138: 325-329. DOI: 10.1016/j.vetmic.2009.03.023

WHO, 2013. World Health Organization.