Antimicrobial resistance genes of *Vibrio parahaemolyticus* and *Aeromonas hydrophila* isolated from Nile tilapia and Mugil fish farms in Kafr-Elsheikh governorate, Egypt.

Ashraf A. Abd El Tawab 1, Fatma I. El-Hofy 1, Gamal R. Hashb-Elnaby 2, Mennat-Allah A. Refaey 2

1 Department of Bacteriology, Immunology, and Mycology, Faculty of Veterinary Medicine, Benha University, Egypt
2 Agriculture Research Center, Animal Health Research Institute, Tanta Branch, Egypt

**ARTICLE INFO**

**ABSTRACT**

The progressive expansion of aquaculture practices led to the development of bacterial disease outbreaks, otherwise, the continuous and extensive use of antibiotics to overcome these diseases. The objective of our study was to investigate the antibiotic susceptibility and antibiotic resistance genes of *Vibrio parahaemolyticus* (*V. parahaemolyticus*) and *Aeromonas hydrophila* (*A. hydrophila*) species isolated from Nile tilapia and Mugil fish farms in Kafr El-Sheikh province, Egypt. A total of 100 clinically diseased fish were bacteriologically examined. The result recorded 65 isolates of *Vibrio* species and *V. parahaemolyticus* was isolated with an incidence of 55.4%. Out of 100 examined fish samples 72 *Aeromonas* species were isolated, *A. hydrophila* was isolated with an incidence of 99.3%. *Vibrio parahaemolyticus* showed high resistance for amoxicillin and colistin followed by cefotaxime and streptomycin. Meanwhile, *A. hydrophila* were highly resistant to amoxicillin and tetracycline followed by streptomycin, cefotaxime, and colistin. Five isolates of *V. Parahaemolyticus* and *A. hydrophila* were screened using PCR for detection of 4 antibiotic resistance genes β-lactamase resistance gene (*blaTEM*); aminoglycosides (*aadA1*); tetracycline-resistant *tetA* (*A*) and polymyxin resistance (*mcr1*) which were distinguished in all five *V. parahaemolyticus* and *A. hydrophila* isolates. The high detection of *V. parahaemolyticus* and *A. hydrophila* antibiotic resistance genes in our study could pose a potential economic problem as it may overlap the control of fish diseases and hence the economy.

1. INTRODUCTION

The speedy expansions of fish culturing and escalating fish requirement results in the extension of aquaculture, increasing stressors on fish, and thus intensify the hazard of diseases (Reverter et al., 2014). Aquaculture is regarded as the major food source that provides a protein of animal source proper for the consumption of the populace in the developing countries (Abbas et al., 2015). Infectious diseases are the chief problem in fish farms, causing huge economic costs due to the serious practices of fish farming (Bulfon et al., 2015). Several *Vibrio* species are well recognized for their severity to cause fish disease, besides, causing mortality in reared fish is very common during early larval stages and can occur suddenly, leading sometimes to the death of the population (Thompson et al., 2004). *Aeromonas hydrophila* is considered a major pathogen producing outbreaks in fish aquaculture with extreme mortality rates; causing severe economic losses to the aquaculture all over the world (Fang et al., 2004). Antibiotics has habitually been collaborated as immersion baths or feed additives to stimulate the fast growth of fish, treat bacterial infections, and also prevent the water plants' growth (Abu Bakar et al., 2010). Multidrug resistance (MDR) developed from the uncontrolled massive antibiotics usage in fish culture to control the bacterial infection and prevent the rapid spread of disease. Besides, the misuse of antibiotics not only improves the antibiotic-resistant bacteria and the spreading of the antibiotic-resistant genes but also results in the existence of antibiotic remains in aquatic animals such as fish (Miranda et al., 2018). Direct transmission of resistant bacteria through food to humans and the transfer of resistance genes to other bacteria happen, thus causing a possible hazard to human wellbeing (Kim et al., 2013). Therefore, this study aimed to study the prevalence of *V. parahaemolyticus* and *A. hydrophila* in some fish farms and to assess the putative risk of possible antibiotic resistance could be transmitted to human through farm fish.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 100 clinically diseased fish samples, 50 Nile tilapia (*Oreochromis niloticus*) and 50 mullet fish (*Mugil cephalus*) were gathered from various fish farms at Kafr el-Sheikh Governorate at the period from January to October (2019). The fish farms were complaining of high mortality rate and fish showed signs of septicemia including unilateral and bilateral exophthalmia, skin ulcers, and
hemorrhages. The examined diseased fish samples were taken in a sterile strong plastic bag with half of its volume pumped with pressurized oxygen and transferred alive with a minimum delay to the bacteriology unit of Animal Health Research Institute, Tanta branch, Egypt for clinical and bacteriological examination. Three hundred and five lesion samples were amassed from 100 diseased fishes; 157 samples from 50 Nile tilapia (O. niloticus) and 148 samples from 50 mullet fish (Mugil cephalus), where the samples were taken from apparently patho-necrotic lesions in liver, kidneys, spleen, heart, anterior intestine, and gills.

2.2. Isolation of Vibrio and Aeromonas species using the conventional cultural method:
The samples were taken by a sterile loopful from the lesions and inoculated in peptone broth 1% (Oxoid for Aeromonas isolation and 1% peptone broth + 3% NaCl for Vibrio isolation and incubated aerobically at 37°C for 18-24 hours. An inoculum from the cultured broth was streaked onto selective diagnostic agar media: Aeromonas selective agar (BSBIG agar, HIMEDIA, M1890-55G) for Aeromonas spp. and Cholera medium TCBS (Oxoid, UK) for Vibrio spp. and incubated for 24 hours at 37°C. One separated typical colony from each selected agar medium was picked up and purified onto the same agar medium. After that one separated typical colony from agar medium was picked up and transferred into the nutrient broth (Oxoid, UK) with 15% glycerol was aerobically incubated at 37°C for 18-24hrs, then preserved in the refrigerator at -85°C (Quinn et al., 2002 and Markey et al., 2013).

2.3. Biochemical identification of Vibrio and Aeromonas isolates
The biochemical identification for the recovered isolated from the examined fish samples were performed according to (Quinn et al., 2002; Nicky, 2004 and Markey et al., 2013) by application of oxidase, catalase, indole production, citrate utilization, urease test, triple sugar iron, and methyl red tests.

2.3. Antibiotic susceptibility testing:
An in-Vitro sensitivity test was done on the isolated V. parahaemolyticus and A. hydrophila strains to study their sensitivity for different antibiotics using the disc diffusion method of Koneman et al. (1997) using different antimicrobial agents (Oxoid, UK): amoxicillin (AM/10), cefotaxime (CTX/30), ciprofloxacin (CIP/5), colistin sulfate (CT/10), gentamicin (GEN/10), streptomycin (S/10) and tetracycline (TE/30). Mueller Hinton broth tubes were inoculated with at least 4-5 colonies of each isolated V. parahaemolyticus and A. hydrophila strains and incubated at 37°C for 24 hrs. Then the plates of Mueller Hinton agar were covered by one ml of the inoculated broth then incubated at 37°C for 24 hrs. The interpretation of results was carried out according to CLSI, (2016).

2.4. Molecular detection of antibiotic resistance genes by the polymerase chain reaction
PCR was used for the detection of antibiotic resistance-associated genes by primers targeting different resistant genes to β-lactams (blaTEM), tetracycline (tetA (A)), aminoglycosides (aadA1), and polymyxin resistant (mcr1) (Metabion, Germany) (Table 1).

The extraction of DNA was performed by QIAamp® DNA Mini Kit (Catalogue no. 51304) according to the manufacturer’s instructions.

The cycling condition for each gene was performed according to the references and Emerald Amp GT PCR Master Mix (Takara, Cat PR310A). The primers were designed at 94°C/ 5 min and the secondary denaturation was occurred at 94°C/ 30 sec for all genes. The annealing process was done at 54°C/40 sec (blaTEM and aadA1), at 50°C/40 sec (tetA (A)) and 60°C/30 sec (mcr1). The extension process was done at 72°C/ 45 sec for all genes except the mcr1 gene at 72°C/ 30 sec. The final extension was occurred at 72°C/ 10 min except the mcr1 gene at 72°C/ 7 min.

The amplification was performed on Eppendorf Master Cycler® (Eppendorf AG, Hamburg, Germany) in a total reaction volume of 25 μl containing 12.5 μl Emerald Amp GT PCR Master Mix, 1 μl of each forward and reverse primers, 4.5 μl molecular biology grade water, and 6 μl test DNA.

The PCR amplicons were analyzed by electrophoresis using a 1.5 % agarose gel in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH = 8.3). A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

Table 1 Oligonucleotide primers and cycling conditions of the primers during conventional PCR

| Target genes | Oligonucleotide sequence (5’ → 3’) | Product size (base pairs) | References |
|--------------|-----------------------------------|--------------------------|------------|
| blaTEM       | F: ATCACCAATAAACCCACG<br>R: CCCGAAAGGTTTTCG | 516                      | Colton et al., 2003 |
|              | F: TATCCAGGTTGTCGCTCA<br>R: GTTCACAGGTGTTTTC | 484                      | Randall et al., 2004 |
| aadA1        | F: GGTCACGCAACAGGC<br>R: CGTCCGACAGGTTAGCG | 576                      |            |
| tetA(A)      | F: CCGTCATGCTGTTGTTTC<br>R: CTTGCGGCTCCTCTGAGG | 308                      | Newton-Foot et al., 2017 |

3. RESULTS
3.1 The prevalence of Vibrio parahaemolyticus in different tissue samples in diseased fishes: Out of 100 diseased fish samples (50 from O. niloticus and 50 from Mugil cephalus), 65 isolates of Vibrio species were isolated and identified, V. parahaemolyticus was isolated with a prevalence of 55.4% (36/65) and isolated from liver, kidneys, spleen, heart, intestine, and gills with a prevalence of 30.5(11/36), 16.7(6/36), 11.1(4/36), 3(3/6) 8.3, 2.8(1/36) and 30.5% (11/36), respectively (Table 2).

3.2. The prevalence of Aeromonas hydrophila in different tissue samples in diseased fishes: Out of 100 diseased fish samples, 72 Aeromonas species isolates from positive samples were identified with a prevalence of 90.3(65/72) and was detected in the liver, kidney, spleen, heart, intestine, and gills with a prevalence of 32.3(21/65),

References

Quinn et al. 2004, and Markey et al. (2013)
Foot et al., 2017
Randall et al. 2004
Newton-Foot et al., 2017
27.7(18/65), 6.2(4/65), 17.0(11/65), 6.2(4/65) and 10.8% (7/65), respectively (Table 2).

3.3. The antibiotic susceptibility tests for the isolated bacteria.

The in-vitro susceptibility tests for the isolated V. parahemolyticus strains (n=36) showed high resistance for amoxicillin 91.7% (33/36) and colistin 63.9% (23/36) followed by cefotaxime 58.3% (21/36) and streptomycin 52.7% (19/36) (Table 3).

The sensitivity tests for the isolated A. hydrophila revealed that the isolated A. hydrophila (n=65) were highly resistant for amoxicillin 100.0% (65/65) and tetracycline 87.7% (57/65) followed by streptomycin 63.1% (41/65), cefotaxime 57.0% (37/65) and colistin sulfate 54.0% (35/65) (Table 4).

### Table 2 Distribution of Vibrio parahemolyticus and Aeromonas hydrophila species isolated from the examined organs.

| Bacterial species | Vibrio parahemolyticus | Aeromonas hydrophila |
|-------------------|-------------------------|----------------------|
| **Fish type**     | **O. niloticus** | **M. cephalus** | **Total** | **O. niloticus** | **M. cephalus** | **Total** |
| Liver             | 4/11 | 30.5 | 8 | 13/21 | 32.3 |
| Kidney            | 5/16 | 16.7 | 5 | 13/18 | 27.7 |
| Spleen            | 3/11 | 11.1 | 0 | 4/4 | 6.2 |
| Heart             | 1/3  | 3.3  | 2 | 8/11 | 7.7 |
| Intestine         | 1/1  | 1.0  | 4 | 0/4 | 0.0 |
| Gill              | 6/11 | 30.5 | 7 | 1/7 | 10.8 |
| Total             | 20/36| 55.4 | 26 | 39/65 | 90.3 |

### Table 3 In-Vitro antimicrobial sensitivity test for isolated V. parahemolyticus strains.

| Antimicrobial agents | Disk concentrations | Sensitive | Intermediate | Resistant |
|---------------------|----------------------|-----------|--------------|-----------|
| Amoxicillin (AML)   | 10 µg                | 3/8.3     | 0/0.0        | 33/91.7   |
| Cefotaxime (CTX)    | 30 µg                | 5/14.0    | 10/27.7      | 21/58.3   |
| Ciprofloxacin (CIP)| 5 µg                 | 11/30.6   | 19/52.8      | 6/16.6    |
| Colistin (CT)       | 10 µg                | 13/36.1   | -            | 23/63.3   |
| Gentamicin (GEN)    | 10 µg                | 27/75.0   | 9/25.0       | 0/0.0     |
| Streptomycin (S)    | 10 µg                | 5/14.0    | 12/33.3      | 19/52.7   |
| Tetracycline (TE)   | 30 µg                | 31/86.1   | 2/5.6        | 3/8.3     |

### Table 4 In-Vitro anti-microbial sensitivity test for isolated A. hydrophila strains.

| Antimicrobial agents | Disk concentrations | Sensitive | Intermediate | Resistant |
|---------------------|----------------------|-----------|--------------|-----------|
| Amoxicillin (AML)   | 10 µg                | 0/0.0     | 0/0.0        | 65/100.0  |
| Cefotaxime (CTX)    | 30 µg                | 13/20.0   | 15/23.0      | 37/57.0   |
| Ciprofloxacin (CIP)| 5 µg                 | 54/83.1   | 9/13.8       | 2/3.1     |
| Colistin sulfate (CT)| 10 µg               | 30/46.0   | -            | 35/54.0   |
| Gentamicin (GEN)    | 10 µg                | 49/75.4   | 11/17.0      | 5/7.6     |
| Streptomycin (S)    | µg                   | 10/15.4   | 14/21.5      | 41/63.1   |
| Tetracycline (TE)   | µg                   | 1/1.5     | 7/45.5       | 57/87.7   |

3.4. Molecular investigation of antibiotic resistance genes in Vibrio parahemolyticus and Aeromonas hydrophila species:

Five random isolates from each Vibrio parahemolyticus and Aeronomas hydrophila were subjected to PCR amplification targeting the antimicrobial resistance determinants β-lactamase (blaTEM), tetracycline resistance (tetA (A)), and aminoglycosides (aadA1) and polymyxin resistant (mcr1) genes which were amplified in all five tested A. hydrophila and all five V. parahemolyticus studied strains giving a product of 516, 576, 484 and 308 bp, respectively. (Figures 1 to 4).

4. DISCUSSION

Intensive aquaculture production leads to the development of infectious disease outbreaks. Bacterial diseases are the most common diseases in intensive fish raising facilities (Kusuda and Salati, 1999).
The present bacteriological examination revealed that *V. parahaemolyticus* were isolated mainly from the liver and gills. Reverse results recorded by Aly et al., (2020) where the highest intensities of *V. parahaemolyticus* which isolated from Gilthead Seabream were mainly from kidneys followed by spleen and liver, this may be due to difference in fish species or season. *Aeromonas hydrophila* was isolated mainly from the liver and kidneys. Nearly similar results were recorded by Enany et al., (2019) and Algammal et al., (2020). The in-vitro sensitivity tests for the isolated *V. parahaemolyticus* showed high resistance for amoxicillin and colistin followed by cefotaxime and streptomycin. These results agreed with those reported by Lee et al., (2018) and Lopatek et al., (2018). In contrast, Xu et al., (2016) reported that most *V. parahaemolyticus* isolates were resistant to streptomycin and Ashrafudoulla et al., (2019) reported that the isolates were highly resistant to tetracycline. These changes may be due to differences in geographical distribution or treatment regimes. The in-vitro susceptibility tests for the isolated *A. hydrophila* showed that the tested *A. hydrophila* strains were highly resistant to amoxicillin may suggest the production of beta-lactamase, which is constant with the findings of Daood, (2012) and Revina et al., (2017). The results of PCR for amplification of blaTEM gene in *V. parahaemolyticus* studies showed that the blaTEM gene was amplified in all 5 *V. parahaemolyticus* studied strains similar to the results reported by Cardoso et al., (2018) and Faja et al., (2019). However, the results were not in agreement with Hu et al., (2020) and Jeamsripong et al., (2020) who failed to detect blaTEM virulent genes in these strains. Meanwhile, the aadA1 and the tetA (A) gene were amplified in all 5 *V. parahaemolyticus* studied strains. These results were agreed with those of Faja et al., (2019). Otherwise, Jiang et al., (2014) cannot detect the tetA gene in any of the isolates. The mcr1 gene was amplified in all 5 *V. parahaemolyticus* studied strains. Lei et al., (2019) firstly reported the occurrence of plasmid-encoded mcr-1 in virulent *V. parahaemolyticus* strain where the mcr-1 gene was detected in one colistin-resistant *V. parahaemolyticus* isolate. The blaTEM gene was amplified in all 5 studied *A. hydrophila* strains, similar results were obtained by Ibrahim (2015) and Okolie (2015). However, the results were not in agreement with (Ndi and Barton, 2011) who failed to detect the blaTEM virulent gene in these strains. The aadA1 gene also was amplified in all 5 *A. hydrophila* strains which agreed with those of Ndi and Barton (2011) and Okolie (2015). The tetA(A) gene was amplified in all 5 *A. hydrophila* studied strains. These results were agreed with those of Ndi and Barton (2011) and Ibrahim (2015). The results were not in agreement with Ighinosa and Okosh, (2012) who failed to detect tet virulent genes in these strains.

5. CONCLUSIONS

Our study exposed an elevated prevalence of *Vibrio* and *Aeromonas* species in aquaculture in the examined farms. The isolated strains displayed a prominent multiple antibiotic resistance associated with high antibiotic resistance genes make aquaculture a reservoir for antibiotic-resistant bacteria which is considered a putative risk on public health and more preventive measures for water pollutant factors must be taken.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

6. REFERENCES

1. Abbas, E.M., Soliman, T., El-Magd, M., Farrag, M., Ismail, R.F., Kato, M., 2017. Phylogeny and DNA barcoding of the family Sparidae inferred from mitochondrial DNA of the Egyptian waters. Journal of Fisheries and Aquatic Science 12, 73-81.
2. Abu Bakar, L, Ayub, M. K., Muhd Yatim, A., Abdallah Sani, N., 2010. Pesticide and antibiotic residues in freshwater
aquaculture fish: chemical risk assessment from farm to table. Asian Journal of Food and Agro-Industry 3, 328–334.
3. Algamal, A. M., Mohamed, M. F., Basma Tawfeik, A., Hozzein, W. N., El Kazzaz, W. M., Mabrok, M., 2020. Molecular typing, antibiogram, and PCR-RFLP based detection of Aeromonas hydrophila complex isolated from Oreochromis niloticus. Pathogens journal 9 (238).
4. Aly, S. M., Eisa, A. A., Noha ElBanna, I., 2020. Characterization of Vibrio parahaemolyticus Infection in Gilthead Seabream (Sparus auratus) Cultured in Egypt. Egyptian Journal of Aquatic Biology and Fisheries 24(1), 553–571.
5. Ashrafuddoula, M., Mizan, M. F. R., Park, H., Byun, K. H., Lee, N., Park, S. H., Ha, S. D., 2019. Genetic relationship, virulence Factors, drug resistance profile, and biofilm formation ability of Vibrio parahaemolyticus isolated from Mussel. Frontiers in Microbiology 10 (513).
6. Bulton, C., Volpatti, D., Galeotti, M., 2015. Current research on the use of plant-derived products in farmed fish. Aquaculture Research 46, 513-51.
7. Cardoso, V. L., Ferreira-Grise, N. M., Mafra, J. F., Duarte, E. A. A., Oliveira, T. A. S., Evangelista-Barreto, N. S., 2018. Genes of virulence and antimicrobial resistance in Vibrio parahaemolyticus prevalent in areas of oestriculture. Boletim do Instituto de Pesca journal 44 (3).
8. CLSI (Clinical and Laboratory Standards Institute), 2016. Performance Standards for Antimicrobial Susceptibility Testing 26th ed. CLSI supplement M100S, Wayne, PA. USA.
9. Coloni, K., Pérez, J., Alonso, R., Fernández-Araza, A., Larino, E., Cistera, R., 2003. Simple and reliable multiplex PCR assay for detection of btaTEM, btaSHV, and btaOXA-1 genes in Enterobacteriaceae. Federation of European Microbiology Societies Microbiology Letters 223, 147-151.
10. Daood, N., 2012. Isolation and antibiotic susceptibility of Aeromonas spp. from the freshwater fish farm and farmed carp (Dam of 16 Tishreen, Lattakia). Damascus University Journal Basic Science 28, 27-39.
11. Enany, M. E., Eidaaroos Abou ELAtta, N. H., M., Eltamimy, N. M., 2019. Microbial causes of summer mortality in farmed fish in Egypt. Suez Canal Veterinary Medical Journal, XXIV (1).
12. Fajah, O. M., Sharad, A. A., Khansa Younis, M., Merriam Alwan, G., Basma Mohammed, J., Asmat Ahmad, 2019. Isolation, detection of virulence genes, antibiotic resistance genes, plasmid profile, and molecular typing among Vibrio parahaemolyticus isolated in Malaysian seawater from recreational beaches and fish. Veterinary World 12 (32).
13. Fang, H.M., Ge, R., Sin, Y.M., 2004. Cloning, characterization, and expression of Aeromonas hydrophila major adhesin. Fish Shellfish Immunology 16, 645-58.
14. Hu, Y., Li, F., Zheng, Y., Jiao, X., Guo, L., 2020. Isolation, Molecular Characterization, and Antibiotic Susceptibility Pattern of Vibrio parahaemolyticus from Aquatic Products in the Southern Fujian Coast, China. Journal of Microbiology and Biotechnology 30(6), 856–867.
15. Ibrahim, L.S.A., 2015. Studies on virulent and antibiotic-resistant genes of Aeromonas species isolated from fish. Ph.D. (Bacteriology, Immunology, and Mycology) Fac. Vet. Med. Suez Canal University. Ighinosia, Isoken, H., Okosh, Anthony, L., 2012. Antibiotic Susceptibility Profile of Aeromonas Species Isolated from Wastewater Treatment Plant. The Scientific Research World Journal 6(10).
16. Jeamsrpong, S., Khant, W., Chuanchuen, R., 2020. Distribution of phenotypic and genotypic antimicrobial resistance and virulence genes in Vibrio parahaemolyticus isolated from cultivated oysters and estuarine water. FEMS Microbiology Ecology 98(6), 70887.
17. Jiang, Y., Yao, L., Li, F., Tan, Z., Zhai, Y., Wang, L., 2014. Characterization of antimicrobial resistance of Vibrio parahaemolyticus from cultured sea cucumbers (Apostichopus japonicas). Letters in Applied Microbiology 59(2),147-54.
18. Kim, M., Kwon, T. H., Jung, S. M., Cho, S. H., Jin, S. Y., Park, N. H., 2013.Antibiotic resistance of bacteria isolated from the internal organs of edible snow crabs.PLoS One8, e70887.
19. Kusuda, R., Salati, F., 1999. Enterococcus seriolicida and Streptococcus iniae in Fish diseases and disorders.Viral, bacterial, and fungal infection of fish, Ed by P.T.K. Woo, and D.W. Bruno vol 3, London, UK: British Library.
20. Lee, I.H., Ab Mutalib, N. S., Law, J. W. F., Wong, S. H., Letchumanan, V., 2018. Discovery on Antibiotic Resistance Patterns of Vibrio parahaemolyticus in Selangor Reveals Carabapenemase Producing Vibrio parahaemolyticus in Marine and Freshwater Fish. Frontiers Microbiology 9, 2513.
21. Li, T., Zhang, J., Jiang, F., He, M., 2019. First detection of the plasmid-mediated colistin resistance gene mcr-1 in virulent Vibrio parahaemolyticus. International Journal of Food Microbiology 308,108290.
22. Lopatek, M., Wieczorek, K., Osek, J., 2018. Antimicrobial Resistance, Virulence Factors, and Genetic Profiles of Vibrio parahaemolyticus from Seafood. Applied Environmental Microbiology 84(16).
23. Markey, B.K., Leonard, F.C., Archambault, M., Cullinan, A., Maguire, D., 2013. Clinical Veterinary Microbiology. Second edition: MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.
24. Miranda, C.D., Godoy, F.A., Lee, M.R., 2018. Current status of the use of antibiotics and the antimicrobial resistance in the Chilean salmon farms. Frontiers Microbiology 9, 1284.
25. Ndi, O. L., Barton, M. D., 2011. Incidence of class 1 integron and other antibiotic resistance determinants in Aeromonas spp. from rainbow trout farms in Australia. Journal of Fish Diseases 34(8), 589–599.
26. Newton-Foot, M.; Snyman, Y.; Maloba, M.R.B., Whetaler, A.C., 2017. Plasmid-mediated mcr-1 colistin resistance in Escherichia coli and Klebsiella spp. Clinical isolates from the Western Cape region of South Africa. Antimicrobial Resistance and Infection Control 6,78.
27. Nicky, B. B., 2004. Bacteria from fish and other aquatic animals (a practical identification manual).CABl publishing is a division of CAB international 106 (85), 83 – 116.
28. Okolie, C.A., 2015. Characterization of antimicrobial resistance genes of Aeromonas spp. isolated from fish and investigation of phytochemical treatment efficacy against resistant isolates. M. Sc. Thesis, School of Life Sciences, University of KwaZulu-Natal Durban.
29. Quinn, P.J., Markey, B. K., Carter, M.E., Donnelly, W.J., Leonard, F. C., 2002. Veterinary Microbiology and Microbial Disease. First Published Blackwell Science Company, Iowa, State University Press.
30. Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L.J.V., Woodward, M.J., 2004. Antibiotic resistance genes, integrons, and multiple antibiotic resistance in thirty-five serotypes of Salmonella enterica isolated from humans and animals in the UK. Journal of Antimicrobial Chemotherapy53, 208–216.
31. Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P., 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy. Current status and future perspectives. Aquaculture 433, 50-61.
32. Revina, O., Avsejenko, J., Cirlle, D., Valdovsk, A., 2017.Antimicrobial resistance of Aeromonas spp. isolated from the sea Trout (Salmo Trutta) in Latvia. Research for rural development.1.
33. Thompson, F.L., Iida, T., Swings, J., 2004. Biodiversity of Vibrios. Microbiology and Molecular Biology Reviews 68, 403-431.
34. Xu, X., Cheng, J., Wu, Q., Zhang, J., Xie, T., 2016.Prevaleance, characterization, and antibiotic susceptibility of Vibrio parahaemolyticus isolated from retail aquatic products in North China. M.C. Microbiology16,32.