INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 24, No. 3, p. 42-49, 2024

RESEARCH PAPER OPEN ACCESS

Antibiotic resistance of *Aeromonas hydrophila* isolated from frog *Hoplobatrachus occipitalis* consumed in Côte d'Ivoire

Blé Yatanan Casimir*[1], Toe Evelyne[2], Zouh Bi Zahouli Faustin[3], Dadié Adjehi[4]

[1]*Université de MAN, UFR Ingénierie Agronomique Forestière et Environnementale, Man, Côte D'Ivoire*

[2]*Université Peleforo Gon Coulibaly, UFR Sciences Biologiques, Département Biochimie-Génétique, Korhogo, Côte D'Ivoire*

[3]*Université Nangui Abrogoua, Centre de Recherche en Ecologie, Abidjan, Côte d'Ivoire.*

[4]*Université Nangui Abrogoua, UFR STA, Laboratoire de Microbiologie, Abidjan, Côte D'Ivoire*

Key Words: *Aeromonas Hydrophila*, Virulence, Antibiotic Resistance, Frog

http://dx.doi.org/10.12692/ijb/24.3.42-49 Article published on March 03, 2024

Abstract

Aeromonas hydrophila is an opportunistic microorganism. It is widely involved in frog infections. *Hoplobatrachus occipitalis* is the species of frog most consumed in the different localities of Côte d'Ivoire. This research aimed to study the extracellular virulence factors and antibiotic resistance profiles of *A. hydrophila* strains isolated from *H. occipitalis*. The determination of extracellular virulence factors were performed onto 113 strains of *A. hydrophila* isolated from frogs *H. occipitalis* using conventional methods. The antimicrobial susceptibility tests were performed by the disk diffusion method using 20 isolates of *A. hydrophila*. All strains isolated produce nuclease (100%) but the ability to produce haemolysins, lipases, amylases and proteases varied from one isolate to another These microorganisms were the most resistant to Amoxicillin (95%), Ampicillin (90%), Tetracyclin (85%), Ceftriaxone (80%) Chloramphenicol (75%). All strains of *A. hydrophila* have developed multi-resistance to different antibiotics used. The multiple antibiotic resistance indices ranged from 0.41 to 0.83. These data show the degree of pathogenicity of the strains isolated from frogs. The consumption of this species could constitute a threat to consume them if hygiene measures are not taken into account.

* **Corresponding Author:** Blé Yatanan Casimir ✉ yatanan12@hotmail.fr

Introduction

Frogs today constitute a very popular food source on the African continent. They are appreciated because of their nutritional characteristics and their taste which is compared to that of chickens (Mohneke *et al.*, 2010). Thus in Burkina Faso, Nigeria and Ivory Coast the consumption rates were respectively 67%, 43% and 55.2% (Mohneke *et al.*, 2010 ; Onadeke *et al.*, 2011; Ble *et al.*, 2016). These frogs are now disappearing due to several diseases, the most important of which is that caused by *Aeromonas hydrophila*. These frogs are disappearing today for causes linked to several diseases.

Aeromonas sp. is part of the microbial flora of the skin and intestines of healthy frogs but can cause disease in these animals. It is responsible for "red leg diseases" and is involved in cases of mass mortality of frogs in captivity and in the natural environment (Forbes *et al.*, 2004). This presence in frogs may also constitute a public health risk for consumers because *A. hydrophila* is an emerging pathogen involved in gastroenteritis, septicemia and endocarditis (Stratev *et al.*, 2012). The areas where frogs are caught are generally shallows, abandoned fish ponds of questionable microbiological quality.

It should be noted that in aquaculture areas, antibiotics are used to prevent and treat bacterial diseases. Furthermore, their use leads to an increase in the prevalence of multi-resistant bacteria which represent health risks (Koudou *et al.*, 2020).

In Côte d'Ivoire, studies have reported the existence of *A. hydrophila* in fish and resistance to antibiotics has also been reported (Kouassi *et al.*, 2018; Kone *et al.*, 2022). Concerning strains of *A. hydrophila* isolated from frogs, there is no data on antibiotic resistance.

Monitoring the resistance of zoonotic bacteria is particularly important to better assess the risks to human health linked to contact with animals or the consumption of food of animal origin. The objective of this study was to investigate the antibiotic resistance profiles of *A. hydrophila* strains isolated from frogs.

Material and methods

Origin of Aeromonas hydrophila isolates

The *A. hydrophila* strains used in this study come from wild frog *H. occipitalis* which were purchased in three local markets in western Côte d'Ivoire: these are ISSIA, DALOA and SINFRA. These strains had been isolated in a previous study using the method of Sarka *et al.* (2012) then stored in the Laboratory at -20°C. Note that the frogs sold on the markets are captured in ponds and shallows. These environments are characterized by a high level of anthropogenic impact, resulting from agricultural and livestock activities in certain cases.

In vitro virulence test of Aeromonas

The search for virulence factors was carried out on 113 strains of *A. hydrophila* distributed as follows: Issia (38 strains), Daloa (38 strains) and Sinfra (37 strains). Five tests were performed in this study.

Hemolytic activity

The hemolytic activity was determined by streak of the strains of *A. hydrophila* onto agar base (Bio-Rad, France) supplemented with 5% sheep blood (Hadeel and Majeed, 2011).

Nuclease activity

Extracellular nucleases (DNases) were determined on DNase agar plates (Difco) from 24-h colonies isolated on alkaline nutrient agar (Castro-Escarpulli *et al.*, 2003).

Proteolytic activity

Casein hydrolysis of *A. hydrophila* were tested on nutritive agar containing 1.5% skimmed (Olaniran *et al.*, 2015).

Lipolytic activity

Lipase activity was carried out by culturing the strains on 0.5% tributyrin agar (Panreac, Barcelona, Spain) emulsified with 0.2% Triton X100 (Castro-Escarpulli *et al.*, 2003).

Amylase activity

Was performed by streak of the isolates onto nutrient agar (Bio-Rad, France) supplemented with 0.4% (W/V) starch in PBS.

Determination of the phenotypic level of antibiotic resistance

A total of 20 isolates of *A. hydrophila* were examined for their resistance to 12 different antibiotics using the disk diffusion method recommended by Bauer *et al.*1966. The antibiotics and concentration ranges tested were as follows: Amoxicillin (AX, 25 µg), Ampicillin (AMP, 10 µg), Cefatoxime (CTX, 30 µg), Ceftazidime (CAZ, 30 µg), Ceftriaxone (CRO, 30 µg), Chloramphenicol (C, 30 µg), Gentamicin (CN, 10 µg), Imipenem (IPM, 10 µg), Kanamycin (K, 30 µg), Oxytetracycline (OT, 30 µg), Streptomycin (S, 10 µg), Tetracycline (TE, 30 µg). The diameter of the inhibition zone was measured and interpreted in accordance with guidelines of the Antibiogram Committee of the French Society of Microbiology. The multiple antibiotic resistances (MAR) index was calculated by dividing the number of antibiotics to which the bacteria were resistant by the total number of antibiotics studied (Sarter *et al.*, 2007).

Data analysis

SPSS 20.0 (IBM Corporation) was used to analyze the data. The MAR index, prevalence and antibiotic resistance of *Aeromonas* isolates were all presented as percentages. The data were analyzed using simple descriptive statistics such as frequency distribution and percentages.

Results

Virulence factor of A. hydrophila isolated from frog

The results presented in Fig. 1 show the pathogenicity of *A. hydrophila* isolated from frogs.

Table 1. Phenotype and MAR index of *A. hydrophila* isolated from frog.

MAR Phenotype	No. of antibiotic showing resistance	Strains code	% strains	MAR Index
AX-AMP-CTX-K-TE-CN-IPM	7	Aeh1	5	0.58 (7/12)
AX-AMP-CAZ-GN-IPM	5	Aeh3	5	0.41 (5/12)
AX-AMP-CRO-C-TE-GN-IPM	7	Aeh2, Aeh10, Aeh12, Aeh17	20	0.58 (7/12)
AX-AMP-CRO-K-TE	5	Aeh5	5	0.41 (5/12)
AX-AMP-CTX-CRO-C-TE-GN	7	Aeh4	5	0.58 (7/12)
AX-AMP-CTX-CRO-K-C-OT-S-TE-GN	10	Aeh14, Aeh18, Aeh20	15	0.83 (10/12)
AX-AMP-K-C-TE-GN-IPM	7	Aeh19	5	0.58 (7/12)
AX-CRO-S-TE-GN-IPM	6	Aeh15	5	0.50 (6/12)
AX-CRO-C-TE-IPM	5	Aeh11	5	0.41 (5/12)
AX-AMP-CTX-C-GN	5	Aeh8	5	0.41 (5/12)
AMP-CRO-C-OT-GN	5	Aeh9	5	0.41 (5/12)
AX-AMP-CRO-C-OT-TE-GN-IPM	8	Aeh16	5	0.66 (8/12)
AX-AMP-CRO-K-C-OT-S-TE-IPM	9	Aeh7, Aeh6	10	0.75 (9/12)
AX-AMP-CRO-S-TE-GN-IPM	7	Aeh13	5	0.58 (7/12)

DNase production materialized by the presence of a clear halo around the colonies (Fig. 2a) was observed in all bacteria (100%). While the proportions of hemolysin (68.42- 91.8%), protease (54.04-68.45%), amylase (47.36-65.78%) and lipase (23.08-47.36%) varied according to the sampling site. The appearance of a clear halo observed in Fig. 2b shows that the strain produces β-hemolysin. Amylase activity is also indicated in Fig. 2c by the presence of a clear zone around the colonies.

Antibiotic resistance of Aeromonas hydrophila isolated

The antibiotic resistance study was carried out on 20 strains of *Aeromonas hydrophila* and is shown in Fig. 3. High resistance has been observed for most β-lactamase class antibiotics such as Amoxicillin (95%), Ampicillin (90%). High resistance was also observed with Tetracycline (85%), Ceftriaxone (80%) and Chloramphenicol (75%). However, low resistance was observed for cephalosporins such as Ceftazidime (5%)

and Cefatoxime (25%). All strains were resistant to Amoxillin except the Aeh9 strain which was susceptible. In addition, the strains showed resistance to at least five antibiotics.

The highest resistance profile was obtained with the Aeh14, Aeh18 and Aeh20 strains which showed resistance to 10 antibiotics out of the 12 tested. A total four *A. hydrophila* isolates (20%) displayed presented the multi-resistance patterns of AX-AMP-CRO-C-TE-CN-IPM and three strains (15%) expressed the patterns of AX-AMP-CTX-CRO-K-C-OT-S-TE-CN. The MAR which was resistance to more than two classes of antibiotics was recorded in all strains of *A. hydrophila*. The multi-resistance index of all isolates was ranged from 0.41 to 0.83 (Table 1).

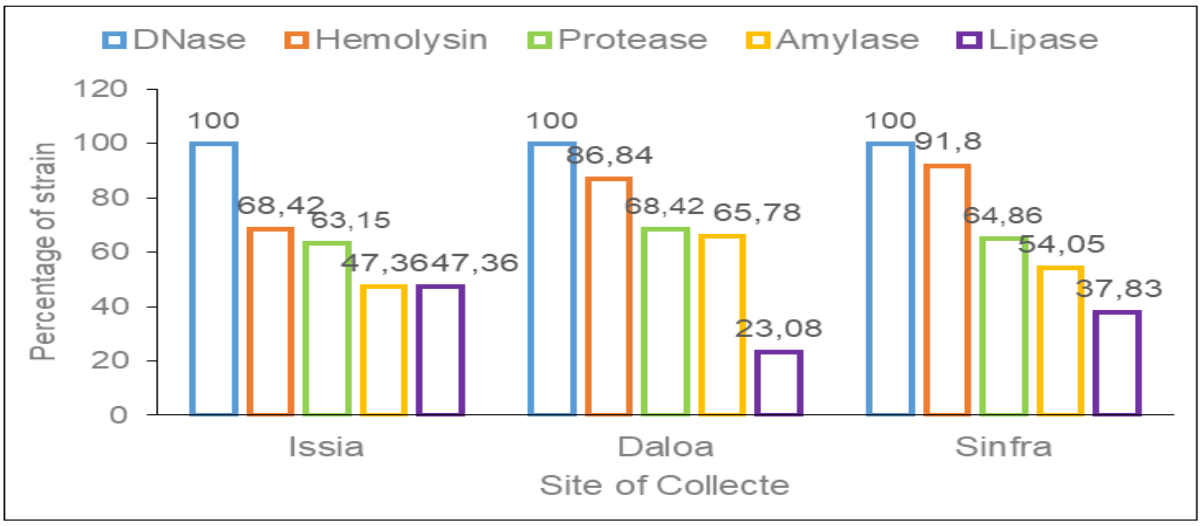

Fig. 1. Virulence level of *A. hydrophila* isolated from *H. occipitalis*.

Discussion

Extracellular virulence factors

In this study, five virulence factors associated with the pathogenicity of *Aeromonas hydrophila* were highlighted. It appears that all strains were positive in the DNAse test (100%) characterized by the formation of the clear zone around the colonies. These results are similar to those of Kone *et al.* (2022) who worked on strains of *A. hydrophila* isolated from fish in Côte d'Ivoire. In addition, a study by Yadav *et al.* (2014) carried out on *A. hydrophila* showed 100% production of the DNAse enzyme. These enzymes can be involved in bacterial nutrition but can also cause diseases in animals and humans (Pemberton *et al.*, 1997). The production of hemolysin by the strains was between 68.42-91.8% depending on the collection sites. This capacity for hemolysin production has already been revealed in several studies, particularly on fish. In fact, the hemolysin production rates were respectively 89.5% and 78.95% in the study of Kone *et al.* (2022) and John *et al.* (2013). This presence of hemolysin in our study shows that these strains are pathogenic. Indeed according to Takahashi *et al.* (2014) hemolysin produced by *A. hydrophila* would be a very important virulence factor. It is considered the main virulence factor of *A. hydrophila*, which exhibits hemolytic and enteric toxicity and is encoded by the ahh1 gene.

The mature, biologically active hemolysin secreted by *A. hydrophila* can bind to and damage the host target cell. This study showed that the isolated strains also produced protease (54.04-68.45%), amylase (47.36-65.78%) and lipase (23.08 - 47.36%). The protease production rate was 72.4% in *A. hydrophila* fish (Kone *et al.*, 2022). Their detection in our strains is an indicator of the pathogenicity of *A. hydrophila* because the protease damages tissues and is involved in the etiology of infections (McMahon, 2000). As for lipases, although they are involved in the nutrition of the bacteria, they alter the structure of the cytoplasmic membrane of the host and intensify its pathogenicity with the presence of the aerolysin gene (Nawaz *et al.*, 2010).

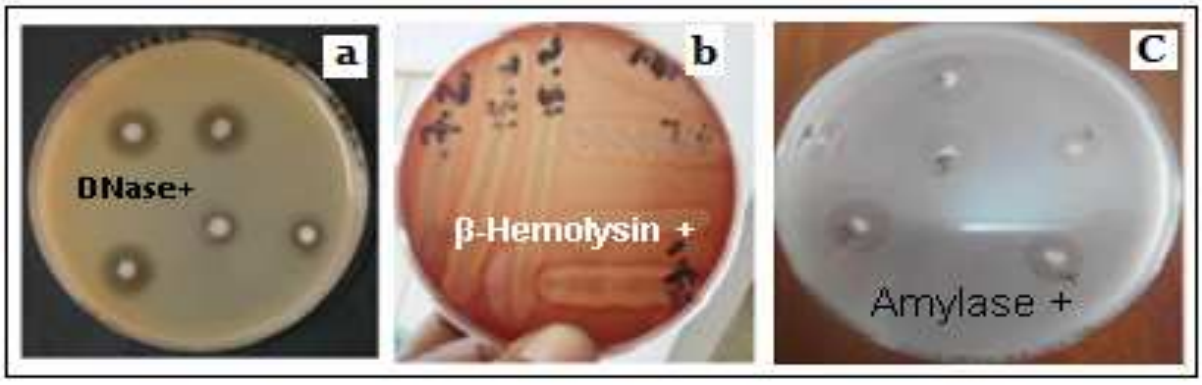

Fig. 2. Some virulence tests of *A. hydrophila* isolated from *H. occipitalis* a: Detection of DNase production on DNase agar, b: detection of hemolysin production of blood agar, c: detection of amylase production on nutrient agar supplemented of 4% starch.

Multi-resistance to antibiotioc of Aeromonas hydrophila

Antibiotic resistance tests carried out on 20 strains of *A. hydrophila* isolated from frogs in this study show that these strains are more resistant to β-lactamases such as Amoxicillin (95%) and Ampicillin (90%). These results confirm the previously reported high resistance of *A. hydrophila* to a wide range of the β-lactam antibiotics family. According to Saavedra *et al.* (2004), strains of *A. hydrophila* develop natural resistance to antibiotics from the β-lactamase family. Contradictory to our finding, high resistance to amoxicillin (98%) and ampicillin (93%) were observed in the work of Koné *et al.* (2022).

The present study showed that Aeromonas strains isolated from frog were characterised by their resistance to TE (85%), CRO (80%), C (75%).

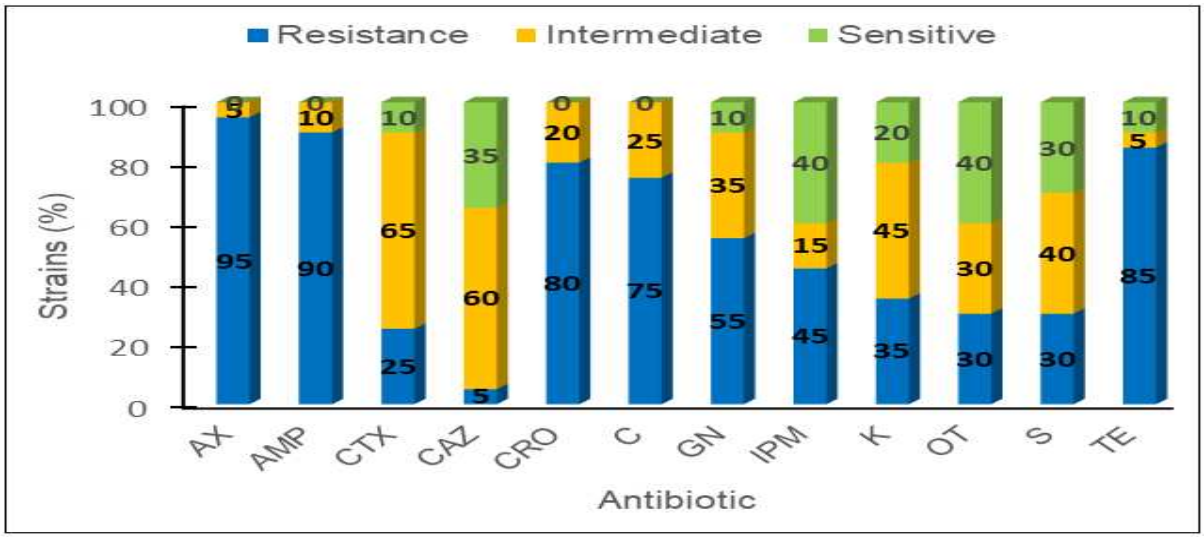

Fig. 3. Frequency of antibiotic resistance of 20 isolates of *A. hydrophila*.

AX : Amoxicillin, AMP : Ampicillin, CTX : Cefatoxime, CAZ : Ceftazidime, CRO : Ceftriaxone, C : Chloramphenicol, GN : Gentamicin, IPM : Imipenem, K : Kanamycine, OT : Oxytetracycline, S : Streptomycin, TE : Tetracycline.

These results confirm previous research that reported high resistance to the antibiotics cited (Elkamouny *et al.*, 2020). The resistance to antibiotics such as GN (55%) and C (75%) obtained in our work are contrary to the results of Rakici *et al.* (2021) who recorded 0% resistance of strains isolated from *Pelophylax* sp frogs. This variation in antibiotic resistance could be linked to the sources of *A. hydrophila*, the frequency

and types of antimicrobials previously used in areas where these animals are captured (Son *et al.*, 1997). Gentamycin resistance (55%) and susceptibility (35%) in this work therefore calls for concern because aeromonads has been largely reported to be sensitive to Gentamycin although there are reports of resistance as well (Jalal *et al.*, 2010). All strains of *A. hydrophila* tested (100%) presented multi-resistance to antibiotics because the MAR index is greater than 0.2 (MAR index > 0.2). These results reveal that the frog represents a source of risky consumption. Highest MAR index (0.83) was observed in three isolates (15%) coded Aeh14, Aeh18 and Aeh20 while low MAR index (0.41) was reported in 25% of strains. Our result is in line with some studies where the capabilities of these bacteria to develop resistance and transmit such effectively have been established. MAR index was between 0.67 and 0.89 in strains of *A. hydrophila* isolated from the fish Clarias gariepinus in Nigeria (Sarka *et al.*, 2017).

Thi *et al.* (2023) also recorded multi-resistance of their strains with a MAR index ranged from 0.33 to 0.92. One of the reasons which could explain this multi-resistance mentioned in our study would be the unsanitary state of the water in which the frogs are caught. In fact, the capture takes place in shallows, puddles, and abandoned fish ponds into which wastewater is continually discharged. Thi *et al.* (2023) established a link between MAR and wastewater discharged into rivers.

Conclusion

The findings of this study revealed that DNAse production is considered as a virulence factor most observed in isolated *Aeromonas* strains followed by hemolysin (68.42-91.8%). In addition, multi-resistance to antibiotics was recorded in all strains with a MAR index value greater than 0.2.

This indicates that the isolates come from high-risk sources such as ponds, ponds, shallows which are areas where frogs are caught. The issue of surveillance frog catching areas must be among our priorities to reduce human contamination.

References

Bauer AW, Kirby WM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology **45**, 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Ble YC, Yobouet BA, Dadié A. 2016. Consumption, proximate and mineral composition of edible frog *Hoplobatrachus occipitalis* from midwest areas of Côte d'Ivoire. African Journal of Science and Research **5(3),** 16-20. https://www.researchgate.net/profile/Adjehi_Dadie/publication/315702287

Castro-Escarpulli G, Figueras MJ, Aguilera-Arreola GL, Soler RE, Fernandez, Aparicio GO, Guarro J, Chacon MR. 2003. Characterisation of Aeromonas spp. Isolated from frozen fish intended for human consumption in Mexico. International journal of Food Microbiology, **84**: 41-49. https://doi.org/10.1016/S0168-1605(02)003.93-8

Elkamouny BM, Rasha ME, Younis GA. 2020. Prevalence and antibiotic resistance of *Aeromonas hydrophila* and Staphylococcus aureus isolated from seafood in Egypt. Mansoura Veterinary Medical Journal **4,** 173-179. http://dx.doi.org/10.35943/mvmj.2020.47607.1009

Forbes MR, David L, McRuer, Rutherford L, Pamela. 2004. Prevalence of *Aeromonas hydrophila* in relation to timing and duration of breeding in three species of Ranid frog. Ecoscience **11,** 282-285. https://www.jstor.org/stable/42901620

Hadeel A, Majeed A. 2011. Characterization of *Aeromonas hydrophila* isolated from patients with Diarrhea. Journal of Kerbala University **9,** 33-42. https://iraqjournals.com/article_19061_0.html

Jalal A, Fatin N, Ashaari MM, John A, Yunus K, Saad S, Omar MN. 2010. Antibiotic resistance microbes in tropical mangrove sediments, East Coast Peninsular Malaysia. African Journal of Microbiology Research **4,** 640-645. https://www.academia.edu/11749653/Antibiotic_resistance_microbes_in_tropical_mangrove_sediments_East_Coast_Peninsular_Malaysia

John N, Hatha AAM. 2013. Distribution, extracellular virulence factors and drug resistance of motile aeromonads in fresh water ornamental fishes and associated carriage water. International Journal of Aquaculture **17(3),** 92-100. http://dx.doi.org/10.5376/ija.2013.03.0017

Koné M, Coulibaly KJ, Yao KE, Vakou SN, Yao KR, Dosso M, N'douba V. 2022. Aeromonas isolated from lagoon Tilapias in Côte D'Ivoire: diversity, distribution and potential virulence. American Journal of Microbiological Research **10(1),** 34-39. https://pubs.sciepub.com/ajmr/10/1/5/index.html

Kouassi AE, Cisse M, Koussemon M, Ouattara A, Gourene G. 2017. Isolation and Identification of *Aeromonas hydrophila*, Pathogen of Farmed Tilapia (*Oreochromis niloticus*) in Region Agneby-Tiassa in Côte d'Ivoire. International Journal of Science and Research **7,** 1914-1918. http://dx.doi.org/10.21275/ART20181068

Koudou AA, Ngazoa SK, Konan KF, Aka E, Addablah A, Coulibaly ND, Sina KSK, Atta DH, Guessend N, Ahoussi S, Dosso M. 2020. Occurrence of multidrug-resistant bacteria in aquaculture farms in Côte d'Ivoire (West Africa). African Journal of Microbiology Research **14(5),** 182-188. http://dx.doi.org/10.5897/AJMR2019.9277

McMahon MAS. 2000. The expression of proteinases and haemolysins by *Aeromonas hydrophila* under modified atmospheres. Journal of Applied Microbiology **89,** 415- 442. http://dx.doi.org/10.1046/j.1365-2672.2000.01129.x

Mohneke M, Onadeko AB, Hirschfeld M, Rödel MO. 2010. Dried or fried: amphibians in local and regional food markets in West Africa. Traffic Bulletin **45,** 193- 202. TRAFFIC BULLETIN 22(3) March 2010 (PDF, 2.5 MB)

Nawaz M, Khan SA, Khan AA, Sung K, Tran Q, Kerdahi K, Steele R. 2010. Detection and characterization of virulence and integrons in *Aeromonas veronii* isolated from catfish. Food Microbiol **27,** 327-331. https://doi.org/10.1016/j.fm.2009.11007

Olaniran AO, Nzimande SBT, Mkize NG. 2015. Antimicrobial resistance and virulence signatures of Listeria and Aeromonas species recovered from treated wastewater effluent and receiving surface water in Durban, South Africa. BioMed Central Microbiology **15,** 15-235. http://dx.doi.org/10.1186/s12866-015-0570-x

Onadeko AB, Egonmwan RI, Saliu JK. 2011. Edible amphibian species: Local knowledge of their consumption in Southwest Nigeria and their nutritional value. West African Journal of Applied Ecology **19,** 67-76. https://www.ajol.info/index.php/wajae/article/view/77567

Pemberton JM, Kidd SP, Schmidt R. 1997. Secreted enzymes of Aeromonas. FEMS Microbiology Letters, **152(1),** 1-10. https://doi.org/10.1111/j.1574-6968.1997.tb10401.x

Rakici E, Altunisik A, Sahin K, Ozgumus OB. 2021. Determination and molecular analysis of antibiotic resistance in Gram-negative enteric bacteria isolated from Pelophylax sp. in the Eastern Black Sea Region. Akademiai Kiado. Acta Veterinaria Hungarica **69(3),** 223-233. https://doi.org/10.1556/004.2021.00039

Saka BA, Adeyemo OK, Odeseye AO. 2017. Multiple antibiotic resistance indices of A*eromonas hydrophila* isolates of muscle of catfish (*Clarias gariepinus*, burchell 1822) from selected markets in Ibadan, Nigeria. African Journal of Microbiology Research **14(5),** 182-188.
http://dx.doi.org/10.5897/AJMR2019.9277

Sarkar A, Saha M, Roy P. 2012. Identification and Typing of *Aeromonas hydrophila* through 16S rDNA-PCR Fingerprinting. Journal of Aquaculture Research and developpment **3(4),** p.
http://dx.doi.org/10.4172/2155-9546.1000146

Sarter S, Nguyen HNK, Hung LT, Lazard J, Montet D. 2007. Antibiotic resistance in Gram-negative bacteria isolated from farmed catfish. Food Control **18,** 1391-1396.
https://doi.org/10.1016/j.foodcont.2006.10.003

Son R, Rusul G, Sahilah AM, Zainuri A, Raha AR, Salmah I. 1997. Antibiotic resistance and plasmid profile of Aeromonas hydrophila isolates from cultured fish, telapia (*Telapia mossambica*). Letters in Applied Microbiology **24,** 479-482.
https://doi.org/10.1046/j.1472-765X.1997.00156.x

Stratev D, Vashin I, Rusev V. 2012. Prevalence and survival of Aeromonas spp. in foods-a review. Revue Médécine Vétérinaire **163,** 486-494.
https://www.academia.edu/22980388/Prevalence_and_survival_of_Aeromonas_spp_in_foods_a_review

Takahashi E, Ozaki, Fujii H, YoshioKobayashi H, Yamanaka H. 2014 "Properties of Hemolysin and Protease Produced by Aeromonas trota", Plos One **9(3),** 2014.
http://dx.doi.org/10.1371/journal.pone.0091149

Thi HHP, Kim DH, Van CTQ, Nguyen PT, Nguyen TL. 2023. Prevalence and antibiotic resistance of *Aeromonas schubertii* causing internal white spot disease on snakehead fish, Channa striata, in the Mekong Delta, Vietnam. Journal of the World Aquaculture Society **54,** 1260-1276.
https://doi.org/10.1111/jwas.12954

Yadav S, Kumar VD, Kumar PP, Kumar DA, Sood N. 2014. Phenotypic and genotypic identification of Aeromonas species from aquatic environment. International Journal of Aquatic Science **5,** 3-20.
https://www.researchgate.net/publication/278764092