**Open Access Library Journal**
2021, Volume 8, e7066
ISSN Online: 2333-9721
ISSN Print: 2333-9705

# Phenotypic Characterization of *Aeromonas hydrophila* Isolates in Fresh Water Fishes in FCT Using Microbact™ GNB 24E Identification Kit

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**How to cite this paper:** Mailafia, S., Nabilah, B. and Olabode, H.O.K. (2021) Phenotypic Characterization of *Aeromonas hydrophila* Isolates in Fresh Water Fishes in FCT Using Microbact™ GNB 24E Identification Kit. *Open Access Library Journal*, **8**: e7066.
https://doi.org/10.4236/oalib.1107066

**Received:** December 7, 2020
**Accepted:** January 23, 2021
**Published:** January 26, 2021

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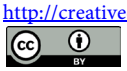

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## Abstract

The study was carried out to determine the phenotypic characters of *Aeromonas* isolates from fresh water fishes sold in FCT using culture and isolation, and conventional biochemical identification method and confirmation with Microbact™ GNB 24E identification system. Out of 400 samples collected from different fishes (257 from *Tilapia zillii*, 77 from *Clarias gariepinus*, 58 from *Lates niloticus*, and 8 from *Alestes nurse*) culture and biochemical characterization revealed that 40 isolates were *Aeromonas* specie and all the isolates (100%) were positive to oxidase, catalase, hydrogen sulphide, voges proskauer and motility tests. Microbact™ GNB 24E kit further revealed 15 out of 40 isolates were *Aeromonas hydrophila*. Descriptive statistics of the results showed an overall prevalence rate of 3.75% with the highest prevalence rate of 6.79% in Bwari Area Council, 3.29% in Abuja Municipal Area Council. *Aeromonas hydrophila* was isolated from all the species of fish in the study area. *Tilapia zillii* had a prevalence rate of 3.11%, while *Lates niloticus* 5.17%, *Clarias gariepinus*, 3.89%, and *Alestes nurse* was 12.50%. Our research was able to isolate *Aeromonas hydrophila* from fresh water fish sold in Federal Capital Territory, which causes zoonotic diseases therefore implies potential danger to man and other animals which makes public health awareness and enlightenment of the dangers associated with *Aeromonas hydrophila* in Nigeria necessary.

## Subject Areas

Veterinary Medicine

## Keywords

Microbact, *Aeromonas hydrophila*, Biochemical, Isolates, Characterization,

Zoonotic, Prevalence

## 1. Introduction

Species of *Aeromonas* are Gram-negative, non-spore-forming, rod-shaped with rounded end measuring 0.3 - 1.0 × 1.0 - 3.5 μm across [1], facultative anaerobic, oxidase-positive, catalase-positive, indole-positive, glucose-fermenting bacteria that are resistant to the 0/129 vibriostatic agent and are chemoorganotrophic [2]. *Aeromonas* species are known as causative agents of a wide spectrum of diseases in man and animals and some motile species are becoming food and waterborne pathogens of increasing importance [3]. *Aeromonas* in fish represent a serious problem and major concern for aquaculturists due to loss of income, reducing global aquaculture development [4]. *Aeromonas* is also associated with food poisoning and some human diseases such as gastroenteritis and extra intestinal symptoms including soft-tissue, muscle infections, septicemia, and skin diseases [5]. *Aeromonas hydrophila* is one of the important pathogens that cause high mortalities in different freshwater fish species in aquaculture [6]. Aeromoniasis is of economic importance because it mainly affects young fish and accounts for epidemic outbreaks which results to massive mortality in different parts of the world [7] and responsible for substantial financial loss to fish farmers worldwide.

Microbact identification system has been used by different researchers such as [8], for the identification of *Aeromonas* species. It is cheaper, is easy and convenient to use than conventional biochemical methods, this is because it is simple and automate the identification of the individual organisms.

According to [9], identification of Aeromonads to genus level can be conducted using routine test employed in the identification of other enteric bacteria. Commercial kit such as, Microbact 24E system have been used in the confirmation of Aeromonads and have been able to identify more than 95% of Aeromonads to genus level when compared with data obtained from PCR method.

Hence the use Microbact 24E identification kit in the confirmation of *Aeromonas* species in this study which is aimed at determining the phenotypic characters of *Aeromonas* species isolated from fresh water fishes in Abuja metropolis. Microbact™ GNB 24 E identification kit was used for the identification of aerobic and facultative anaerobic gram-negative bacteria. The kit contained 24 biochemical substrates, lysine, ornithine, hydrogen sulphide, glucose, mannitol, xylose, ONPG, indole, urease, Voges proskauer, citrate, tryptophan, gelatin, malonate, inositol, sorbitol, rhamnose, sucrose, lactose, arabinose, adonitol, raffinose, salicin, arginine.

## 2. Materials and Method

### 2.1. Study Area

The research work was carried out in 2019 in Federal Capital Territory (FCT).

Federal Capital Territory (FCT) was formed in 1976. The territory shares boundary with Kogi, Kaduna, Niger and Nassarawa lying between latitude 8.25˚N and 9.20˚N of the equator and longitude 6.45˚E and 7.39˚E east of Greenwich Meridian, Abuja is geographically located in the center of the country. The Federal Capital Territory has a landmass of approximately 7315 $km^2$ with an estimated population of 979,876. The territory is made up of six area councils, namely: Abaji, Abuja Municipal, Gwagwalada, Kuje, Bwari, and Kwali [10].

### 2.2. Sample Collection and Transportation

About 400 fish samples were purchased from different markets in the three area councils (Bwari, Abuja municipal, and Gwagwalada). 245 samples were purchased from five markets within AMAC, 103 were purchased from 3 markets within Bwari Area Council, and 52 samples were purchased from 2 markets within Gwagwalada Area Council. The fish samples were transported aseptically in ice-packed containers to Public Health Bacterial Zoonosis Laboratory of Faculty of Veterinary Medicine, Ahmadu Bello University Zaria for examination.

### 2.3. Sample Processing

The collected fish sample was kept in dorso ventral position and the abdomen was then washed with 70% ethanol to reduce the number of incidental organism. A deep ventral incision was made and transverse incision was conducted to expose the visceral organ using a sterilized scissors, scapel and forcep, 5 g of fish intestine was weighed and introduced into 45 mls of peptone water as pre enrichment media, and then stomached using a stomacher for about 2 minutes to homogenize the sample, after which 1 ml was taken and transferred into labeled test tubes containing 9 mls of already prepared alkaline peptone water or brain heart infusion broth and covered quickly and then incubated at 37˚C for 24 hours [11].

### 2.4. Media Preparation

Agars were prepared based on the manufacturer's instruction (Oxoid England, and Hi media India).

### 2.5. Enrichment

Brain heart infusion broth was used for enrichment. 37 grams was suspended in 1 litre of distilled water, boiled to completely dissolve and 5 mls were dispensed into each enrichment bottle autoclaved at 121˚C for 15 minutes and allowed to cool. 1 ml from the pre enrichment was transferred to 9 mls of brain heart infusion broth and then incubated at 37˚C for 18 hours.

### 2.6. Selective Plating

A loopful of the inoculum from the incubated test tubes of brain heart infusion broth was taken and streaked on Ampicillin sheep blood agar plates, the plates

were incubated at 37˚C for about 18 - 24 hours and smooth, convex, grayish colonies was isolated which is typical of *Aeromonas* species.

### 2.7. Preliminary Identification

Preliminary screening and identification were conducted using gram staining, oxidase test and catalase test. Gram negative, oxidase and catalase positive isolates was stored on blood agar slant for further biochemical characterization [12].

### 2.8. Conventional Biochemical Characterization

Biochemical test such as hydrogen sulphide, motility test, indole test, methyl red test, citrate test, DNase test, urease test, Voges proskauer test, sugars (glucose, inositol and mannitol, was performed [12].

### 2.9. Confirmation of *Aeromonas* to Specie Level Using Microbact™ GNB 24 E Identification Kit

#### 2.9.1. Preparation of Inoculum

A single colony of post 18 - 24 hour's culture was picked using an inoculating loop and then emulsified in 5 mls of already prepared normal saline (0.85 g into 100 mls of distilled water and allowed to dissolve before been autoclaved), and then mixed thoroughly to get a homogenous suspension [13].

#### 2.9.2. Inoculation

The plates were numbered first and the well of individual substrate was exposed by peeling its back. The plates were then placed on the holding tray. A sterile micro pipette was used to add 100 ul of bacterial suspension to each well. One drop of mineral oil was then added to well 1, 2, and 3 as shown in **Figure 1**. The inoculated rows was resealed with adhesive seal and incubated at 37˚C for 18 - 24 hours. After incubation the strips was removed from the incubator and the sealing tape was pealed backward. As shown in **Figure 2**, for well 8 (indole production) 2 drops of kovac's reagent was added and the result was read after 2 minutes of the addition of the reagent. For well 10 (voges proskauer reaction) one drop of VP1 and VP2 reagent was added and the result was read after 15 - 30 minutes. For well 12 (Tryptophan Deaminase) one drop of the TDA reagent was be added and the result was read immediately. For well 7 (ONPG), after reading the result, one drop of nitrate A and nitrate B reagent was added to the well. Production of red colour indicates that nitrate have been reduced to nitrite ($NO_2$). For wells that exhibited a yellow colour after the addition of nitrate reagents, Zinc powder was added to the well to determine if nitrate was reduced completely to nitrogen gas ($N_2$).

#### 2.9.3. Interpretation

The codes gotten from the reaction was entered into Microbact computer aided identification package software and the resultant identity and percentage probability of the organism was recorded.


**Figure 1.** Microbact™ GNB 24E kit after the addition of bacterial suspension in each well before incubation.


**Figure 2.** Microbact™ GNB 24E after incubation at 37˚C for about 18 - 24 hours and addition of required reagent. Now ready for reading using Microbact chart.

## 3. Result

Table 1 showed the number of fishes purchased from different area councils in FCT Abuja. The highest number of 129 *Tilapia Zillii,* was purchased from AMAC, while 92 *Tilapia zillii* was purchased from Bwari area council and 36 *Tilapia zillii* from Gwagwalada area council, making a total of 257 *Tilapia zillii*. The highest *Clarias gariepinus* was purchased from AMAC which was about 70 and 7 was

sourced from Gwagwalada area council respectively, no *Clarias gariepinus* was purchased from Bwari area council. 40 *Lates niloticus* were purchased from AMAC and 9 each was purchased from Bwari and Gwagwalada area council, making a total of 58 *Lates niloticus.* 6 *Alestes nurse* were purchased from AMAC and 2 from Bwari area council making a total of 8 *Alestes nurse.*

Table 2 showed the characteristics of various complete biochemical reaction of 40 isolates suspected to be *Aeromonas* specie out of 400 fish samples used based on morphology, gram staining and complete conventional biochemical test. All the 40 isolates were gram negative, greyish, moist, circular, and raised on Ampicillin sheep blood agar. All 40 isolates were positive for oxidase, catalase, hydrogen sulphide production, voges proskauer, and motility as 39 isolates further fermented glucose, while 38 isolates fermented mannitol. Urease production was positive for 37 isolates, methyl red was positive for 34 isolates, 24 isolates were also positive for indole and 18 isolates were positive for inositol.

Table 3 showed that 15 isolates were confirmed as *Aeromonas hydrophila* based on Microbact™ GNB 24E identification system. All the 15 isolates were positive for motility, nitrate, lysine, hydrogen sulphide, glucose, sucrose, arginine while 14 of the isolates reacted to ornithine, mannitol, Ortho-Nitrophenyl *β*-galactoside (ONPG), Urease and voges proskauer. Twelve (12) isolates were positive to xylose and rhamnose. Eleven (11) isolates were positive to citrate. 9

**Table 1.** Fishes purchased from different area councils in FCT.

| Types of Fish | AMAC | Bwari | Gwagwalada | Total |
|---|---|---|---|---|
| ***Tilapia zillii*** | 129 | 92 | 36 | 257 |
| ***Clarias gariepinus*** | 70 | 0 | 7 | 77 |
| ***Lates niloticus*** | 40 | 9 | 9 | 58 |
| ***Alestes nurse*** | 6 | 2 | - | 8 |
| **Total** | **245** | **103** | **52** | **400** |

**Table 2.** Biochemical reactions of the 40 suspected isolates of *Aeromonas* specie.

| Biochemical test | No. of Positive | No. of Negative |
|---|---|---|
| Oxidase | 40 | 0 |
| Catalase | 40 | 0 |
| $H_2S$ | 40 | 0 |
| VP | 40 | 0 |
| Motility | 40 | 0 |
| Glucose | 39 | 1 |
| Mannitol | 38 | 2 |
| Urease | 37 | 3 |
| Citrate | 36 | 4 |
| Methylred | 34 | 6 |
| Indole | 24 | 16 |
| Inositol | 18 | 12 |

**Table 3.** Biochemical reaction of 15 isolates confirmed as *Aeromonas hydrophila* using Microbact™ GNB 24E Test kit.

| Biochemical | Positive | Negative |
|---|---|---|
| Motility | 15 | 0 |
| Nitrate | 15 | 0 |
| Lysine | 15 | 0 |
| Ornithine | 14 | 1 |
| $H_2S$ | 15 | 0 |
| Glucose | 15 | 0 |
| Mannitol | 14 | 1 |
| Xylose | 12 | 3 |
| ONPG | 14 | 1 |
| Indole | 2 | 13 |
| Urease | 14 | 1 |
| VP | 14 | 1 |
| Citrate | 11 | 4 |
| TDA | 0 | 15 |
| Gelatin | 3 | 12 |
| Mannose | 7 | 8 |
| Inositol | 1 | 14 |
| Sorbitol | 9 | 6 |
| Rhamnose | 12 | 3 |
| Sucrose | 15 | 0 |
| Lactose | 8 | 7 |
| Arabinose | 7 | 8 |
| Adonitol | 1 | 14 |
| Raffinol | 7 | 8 |
| Sal | 9 | 6 |
| Arginine | 15 | 0 |

isolates were positive to Sorbitol and salicine. 8 isolates were positive to lactose. 7 isolates were positive to Arabinose, raffinol, and mannose. 3 isolates were positive for gelatin, 2 isolates were also positive to indole and 1 isolate was positive for adonitol and inositol. No isolate was positive for Tryptophan deaminase (TDA).

Table 4 showed the prevalence of *Aeromonas hydrophila* in different area councils of FCT, Abuja. The prevalence of *Aeromonas hydrophila* from fishes purchased in Bwari was the highest (6.79%) when compared to that obtained from fishes purchased in AMAC (3.29) and Gwagwalada had no report of *Aeromonas hydrophila*. The overall prevalence of *Aeromonas hydrophila* isolates from the 400 fishes purchased in Federal Capital Territory 3.75%.

Table 5 showed the percentage distribution of *Aeromonas hydrophila* among fishes purchased in Bwari. The highest isolation rate is for *Alestes nurse* was (50%) followed by *Lates niloticus* (22.20%) and *Tilapia zillii* having the lowest (4.34%), no isolation was obtained for *Clarias gariepinus* with the total isolation rate of 6.79% for *Aeromonas hydrophila* in Bwari.

Table 6 showed the percentage distribution of *Aeromonas hydrophila* among fishes purchased from AMAC. *Clarias gariepinus* had the highest isolation rate (4.28%) followed by *Tilapia zillii* (3.10%) and then *Late niloticus* (2.50%). No isolation rate was recorded for *Alestes nurse.*

## 4. Discussion

### 4.1. Isolation of *Aeromonas* Species

*Aeromonas* species are capable of growing on the common culture media used in the clinical laboratory, such as Sheep Blood Agar (SBA), as well as Chocolate Agar. The organism is also known to produce hemolysis on sheep blood agar, which is indicated by our findings. The colonies were found to be round, raised opaque, with entire edge, smooth, and often had mucoid surface [14]. The organism was known to grow on common culture media used for the isolation of Enteric bacteria, such as MacConkey Agar, XLD Agar, and Hektoen Enteric

Table 4. Prevalence of *Aeromonas hydrophila* in different area councils of FCT, Abuja.

| Area councils | Numbers of fishes used | Number of isolates | % Prevalence |
|---|---|---|---|
| Bwari | 103 | 7 | 6.79 |
| AMAC | 245 | 8 | 3.26 |
| Gwagwalada | 52 | 0 | 0.00 |
| **Total** | **400** | **15** | **3.75** |

Table 5. Percentage distribution of *Aeromonas hydrophila* among fishes in Bwari.

| Types of fishes | Number of fishes | Number of isolates | Prevalence rate % |
|---|---|---|---|
| ***Tilapia zillii*** | 92 | 4 | 4.34 |
| ***Lates niloticus*** | 9 | 2 | 22.20 |
| ***Alestes nurse*** | 2 | 1 | 50.00 |
| ***Clarias gariepinus*** | 0 | 0 | 0.00 |
| **Total** | 103 | 7 | 6.79 |

Table 6. Percentage distribution of *Aeromonas hydrophila* among fishes in AMAC.

| Types of fish | Number of fish | Number of isolates | Prevalence rate % |
|---|---|---|---|
| ***Tilapia zillii*** | 129 | 4 | 3.10 |
| ***Clarias gariepinus*** | 70 | 3 | 4.28 |
| ***Lates niloticus*** | 40 | 1 | 2.50 |
| ***Alestes nurse*** | 6 | 0 | 0.00 |
| **Total** | 245 | 8 | 3.26 |

Agar. It also grows on Cefsulodin-Irgasan-Novobiocin (CIN) plate, which is initially formulated for the *Yersinia enterocolitica*, and forms pink centered olonies on that medium [14]. Another culture medium for the selective isolation of *Aeromonas* is ampicillin blood agar, which is used for its recovery from feces, and contains 20 μg/ml of ampicillin [15].

400 fish samples were used to carry out the research work. Pre enrichment was first done using peptone water, after which enrichment was done using brain heart infusion broth. It was then cultured on Ampicillin sheep blood agar, which is the widely used medium for the isolation of *Aeromonas* specie is ampicillin sheep blood agar and [16], noted that *Aeromonas* have been regarded as being universally resistant to penicillin therefore ampicillin was incorporated the culture media for the selective isolation of aeromonads which produced a grayish, circular, raised, beta hemolytic colonies. Ampicillin sheep blood agar (ASBA) have also been recommended by [17], for the selective cultivation of *Aeromonas* species. Our study was able to isolate *Aeromonas hydrophila* from fresh water fishes using Ampicillin Sheep Blood Agar, which grew at 37˚C. All isolates were beta hemolytic, round, raised, and smooth, which is concurred with [11], who noted that *Aeromonas* species are usually hemolytic, round, raised, smooth and often mucoid on sheep blood agar.

### 4.2. Biochemical Characteristics

The series of biochemical profiles such as oxidase test, catalase, citrate test, methyl red test, voges proskauer, motility, indole, hydrogen sulphide, and sugar fermentation tests such as inositol, glucose and mannitol indicated quantitative phenotypic reactions typical of *Aeromonas* species. The test that yielded high percentage of positivity included 40 isolates which were all gram negative, oxidase and catalase positive, motile, citrate positive, voges prokauer positive, produced hydrogen sulphide, glucose positive and very few negative, inositol negative and positive, mannitol positive and few negative, urea positive and few negative were suspected to be *Aeromonas.*

Biochemiocal characterization conducted in our studies revealed 40 isolates that were identified as *Aeromonas* isolates. [18], found *Aeromonas* stains in 29 (13.18%) of samples from diarrhoeic humans from Zaria through conventional biochemical studies which is at variance with our studies. As the years go by, new methods always emerge for the identification of *Aeromonas* as apart from biochemical methods. This is attested to the fact that biochemical methods tend to be cumbersome and there are chances of development of false positive results. Despite all odds biochemical tests remain one of the best methods for preliminary and conventional identification of *Aeromonas* starins from contaminated samples. Furthermore, biochemical could serve as a prelude for applications of rapid test methods in confirmation of field isolates. The knowledge and skills for the identification of prevailing biotypes of *Aeromonas* in fish could be necessary to formulate startegies for the control of *Aeromonas* in a population.

### 4.3. Microbact™ GNB Identification System

*Aeromonas* identified by the conventional biochemical test reaction were subjected to additional analytical profile index test using Microbact™ 24E GNB kit (Oxoid, England) which is a commercial biochemical kit in micro plate format for identifying Enterobacteriaceae and Miscellaneous gram-negative bacilli. Organism identification is based on pH change and substrate utilizations as established by published reference methodologies [14]. The kit has Microbact™ computer aided identification package that is consulted for the identification choices. The percentage figure shown against the organism name is the percentage share of the probability for that organism as a part of the total probabilities for all choices. It has been used by different researchers such as [8], for the identification of *Aeromonas* species. It is cheaper, is easy and convenient to use than conventional biochemical methods, this is because it is simple and automate the identification of the individual organisms.

According to [9], identification of aeromonads to genus level can be conducted using routine test employed in the identification of other enteric bacteria. Commercial kit such as, Microbact 24E system have been used in the confirmation of Aeromonads and have been able to identify more than 95% of Aeromonads to genus level when compared with data obtained from PCR method.

### 4.4. Prevalence

The prevalence rate of *Aeromonas hydrophila* in Federal Capital Territory may be associated with environmental factors (The growth of microorganisms is greatly affected by the chemical and physical nature of their surroundings such as solutes and water activity, pH, temperature, oxygen level, pressure and radiation). Isolation method, such as the type of Agar used, Season of the year. A study conducted by [19], from north Africa also found out that the isolation of *Aeromonas* specie to be highest during the months of winter and lowest during summer.

The overall prevalence rate of *Aeromonas hydrophila* isolated from different fresh water fishes purchased from different markets within FCT is 3.75% which is lower that the prevalence rate found by [6], which was 3.92%. This may be due to the type of sample used as the sample used which was stool. It was also lower than the prevalence rate of *Aeromonas hydropila* from diarrheic reported by [18], which was 6.81%. it was also lower that [20], who had 91% prevalence of *Aeromonas hydrophila* from boreholes used for drinking in Maiduguri, the high prevalence rate might be due to the type of sample used by [20]. The prevalence of *Aeromonas hydrophila* from fishes purchased in Bwari was the highest at (6.79%) when compared to the prevalence of *Aeromonas hydrophila* from fishes purchased in AMAC (3.29) and Gwagwalada had no prevalence recorded.

The distribution of aeromoniasis in federal capital territory showed that *Alestes nurse* had the highest prevalence of aeromoniasis in Bwari (50%) and *Clarias gariepinus* had the highest prevalence (4.28%) in Abuja Municipal Area

Council (AMAC). All the species showed varying prevalence in different fishes and different areas of the Federal Capital Territory. This is in agreement with previous workers [20]. This clearly shows variations in infection rates in different areas. Zero prevalence encountered in Gwagwalada and in *Alestes nurse* in Abuja Municipal Area Council (AMAC) may be attributed to small sample size, system of production, season of the year, or other geographical factors.

## 5. Conclusion

This study demonstrated the isolation and confirmation of *Aeromonas hydrophila* using both conventional method and Microbact™ 24E identification kit with an overall prevalence of 3.75%. Since *Aeromonas hydrophila* is indicated in human infection, the prevalence in fish may be a potential source of human infection especially were fish and fish products are not properly handled before consumption. There is therefore need for public health awareness and enlightenment of the dangers associated with *Aeromonas hydrophila* in Nigeria.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Parker, J.L. and Shaw, J.C. (2011) Aeromonas Species. Clinical Microbiology and Disease. *Journal of Infection*, **62**, 109-118. https://doi.org/10.1016/j.jinf.2010.12.003

[2] Igbinosa, I.H., Igumbor, E.I., Aghdasi, F., Mvuyo, T. and Okoh, A.I. (2012) Emerging Aeromonas Species Infections and Their Significance in Public Health. *The Scientific World Journal*, **2012**, Article ID: 625023. https://doi.org/10.1100/2012/625023

[3] Ansari, M., Rahimi, E. and Raissy, M. (2011) Antibiotic Susceptibility and Resistance of Aeromonas Species Isolated from Fish. *African Journal of Microbiology Research*, **5**, 5772-5775. https://doi.org/10.5897/AJMR11.1240

[4] Lafferty, K.D., Harvell, C.D., Conrad, J.M., Friedman, C.S., Kent, M.L., Kuris, A.M., Powell, E.N., Rondeau, D. and Saksida, S.M. (2015) Infectious Diseases Affect Marine Fisheries and Aquaculture Economics. *Annual Review of Marine Science*, **7**, 471-496. https://doi.org/10.1146/annurev-marine-010814-015646

[5] Batra, P., Mathur, P. and Misra, M.C. (2016) Aeromonas spp.: An Emerging Nosocomial Pathogen. *Journal of Laboratory Physicians*, **8**, 1-4. https://doi.org/10.4103/0974-2727.176234

[6] Mailafia, S., Nafarnda, W. and Sugun, M.Y. (2017) Occurrence and Antimicrobial Susceptibility Patterns of *Aeromonas hydrophila* Isolates among Diarrhoeic Patients from University of Abuja Teaching Hospital Nigeria. *Journal of Pure and Applied Microbiology*, **11**, 63-70. https://doi.org/10.22207/JPAM.11.1.09

[7] Ahamad, B., Punniamurthy, D., Kumar, N.S., Malmarugan, V., Suresh, R. and Ranganathan, V. (2013) Outbreak of Bacterial Haemorrhagic Septicaemia in Freshwater Carps in Thanjavur Region of Tamil Nadu. *Proceedings of the National Seminar on Current Perspectives in Biological Sciences*, 121-151.

[8] Dashe, Y.G., Raji, M.A., Abdu, P.A., Oladele, B.S. and Olarinmoye, D. (2014) Isola-

tion of *Aeromonas hydrophila* from Commercial Chickens in Jos Metropolis, Nigeria. *International Journal of Poultry Science*, **13**, 26-30. https://doi.org/10.3923/ijps.2014.26.30

[9] Ghenghesh, K., Ahmed, S., El-Khalek, R., Al-Gendy, A. and Klena, J. (2008) Aeromonas-Associated Infections in Developing Countries. *The Journal of Infection in Developing Countries*, **2**, 81-98. https://doi.org/10.3855/T2.2.81

[10] Olabode, H.O.K., Mailafia, S., Adah, B.M.J., Nyambee, P. and Bello, R.H. (2011) Antibiogram of Bacterial Isolates Associated Reproductive Abnormalities in Sheep in Gwagwalada-FCT, Nigeria. *Journal of Agriculture and Veterinary Science*, **3**, 20-27.

[11] Omeje, V.O. and chukwu, C.C. (2015) Prevalence of *Aeromonas hydrophila*. *Journal of Antimicrobial Chemotherapy*, **46**, 297-230.

[12] Cheesbrough, M. (2006) District Laboratory Practice: In Tropical Countries. Part 2, 2nd Edition, Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511543470

[13] Burke, V., Robinson, J., Atkinson, H.M. and Gracey, M. (1982) Biochemical Characteristics of Enterotoxigenic Aeromonas Specie. *Journal of Clinical Microbiology*, **15**, 48-52. https://doi.org/10.1128/JCM.15.1.48-52.1982

[14] Horneman, A.J. and A Josko, A. (2011) Vibrio, Aeromonas, Plesiomonas and Campylobacter Species. In: Mahon, C.R., Lehman, D.C. and Manuselius, G., Eds., *Textbook of Diagnostic Microbiology*, 4th Edition, W.B. Sanders Company, Philadelphia, Chapter 20, 462-481.

[15] De la Maza, L.M., *et al.* (2013) Aeromonas and Pleisiomonas. In: *Color Atlas of Medical Bacteriology*, 2nd Edition, ASM Press, Washington DC, Chapter 15, 131-135.

[16] Rey, G., Fouillet, A., Bessemoulin, P., Frayssinet, P., Dufour, A., *et al.* (2009) Heat Exposure and Socio-Economic Vulnerability as Synergistic Factors in Heat-Wave-Related Mortality. *European Journal of Epidemiology*, **24**, 495-502. https://doi.org/10.1007/s10654-009-9374-3

[17] Mishra, S., Balakrish, G., Bhadra, K.R., Sikdar, N.S. and Pal, C.S. (1987) Comparison of Selective Media for Primary Isolation of Aeromonas Species from Human and Animal Feaces. *Journal of Clinical Microbiology*, **25**, 2040-2043. https://doi.org/10.1128/JCM.25.11.2040-2043.1987

[18] Malafia, S., Ajogi, I. and Nok (2008) Occurrence and Epidemiology of Aeromonas Infections Isolated from Diarrhoeic Patients in Zaria, Nigeria. *Vom Journal of Veterinary Science*, **5**, 27-30.

[19] Ghenghesh, K.S., El-Ghodban, A., Dkakni, R., Abeid, S., Altomi, A., Tarhuni, A. and Marialigeti, K. (2001) Prevalence, Species Differentiation, Haemolytic Activity, and Antibiotic Susceptibility of Aeromonads in Untreated Well Water. *Memórias do Instituto Oswaldo Cruz*, **96**, 169-173. https://doi.org/10.1590/S0074-02762001000200006

[20] Bello, H.S., Mustapha, P.A., Ismail, H.Y., Isa, M.A. and Mangga, H.K. (2016) Detection of Aeromonas Species from Water Sources in North Eastern Nigeria. *International Journal of Innovative Science, Engineering & Technology*, **3**, 140.