Scientific Research Publishing

Advances in Microbiology, 2023, 13, 249-265
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Phenotypic and Antimicrobial Susceptibility Studies of *Proteus mirabilis* Isolates from Fresh Water Fishes in FCT, Abuja-Nigeria

Victor Bitrus Shammah[1*], Samuel Mailafia[1], James Agbo Ameh[1], Casmir Ifeanyichukwu Cejetan Ifeanyi[1], Adetola Opeyemi Adebari[2], Ebenezer Odey Odey[1], Reece Asoloko Sabo[3]

[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria
[2]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria
[3]Department of Molecular Biotechnology, Jagiellian University, Krakow, Poland
Email: *bitrusshammah0@gmail.com

How to cite this paper: Shammah, V.B., Mailafia, S., Ameh, J.A., Ifeanyi, C.I.C., Adebari, A.O., Odey, E.O. and Sabo, R.A. (2023) Phenotypic and Antimicrobial Susceptibility Studies of *Proteus mirabilis* Isolates from Fresh Water Fishes in FCT, Abuja-Nigeria. *Advances in Microbiology*, **13**, 249-265.
https://doi.org/10.4236/aim.2023.135016

Received: April 17, 2023
Accepted: May 27, 2023
Published: May 30, 2023

Abstract

Our study was carried out to determine the phenotypic characterization antimicrobial susceptibility of *Proteus mirabilis* from fish in FCT, Abuja using isolation, selective plating, preliminary observation, complete biochemical method and antimicrobial susceptibility testing. The biochemical tests conducted includes include Citrate Utilization test, Triple Sugar Iron test, Urea test, Methyl Red test, Indole test, and Voges Proskauer test. The isolates were confirmed by Microbact^{TM}GNB24E identification kit (Oxiod, UK). A total of 400 fish samples were bought in the market from three area council of the FCT. The result of the study showed overall prevalence rate of (13) 3.25% of *Proteus mirabilis* isolates. Distribution based on Area councils showed that AMAC had higher prevalence rate of 4.81%, while Bwari had 2.99% and Gwagwalada with 2.57% prevalence. All isolates were subjected to antimicrobial susceptibility testing using the modified single disc diffusion method. From the antimicrobial susceptibility testing done it was discovered that *Proteus mirabilis* are resistant to Amoxyclav (100%), Erythromycin (92.3%), Tetracycline (92.3%) and Ceftriaxone (23.1%). However, the isolates were susceptible to Ofloxacin (100%), Netillin (92.3%), Levofloxacin (92.3%), Ceftazidime (76.9%), Co-trimoxazole (69.2%) and Gentamicin (61.5%). Since *Proteus mirabilis* sources of zoonotic diseases and can potentially be dangerous to humans and other animals, our research was able to isolate it from fresh water fish sold in the Federal Capital Territory. This makes public health

awareness of the risks associated with *Proteus mirabilis* in Nigeria necessary.

Keywords

Proteus mirabilis, Fishes, Isolation, Microbact^{TM}GNB24E, Biochemical, Characterization, Prevalence

1. Introduction

Proteus species belong to the *Enterobacteriaceae* family [1]. The name "*Proteus*" refers to their pleomorphism, after the Greek God Proteus who could assume any shape [2]. *Proteus mirabilis* is an opportunistic pathogen, a part of the *Enterobacteriaceae* family, small gram-negative bacilli and facultative anaerobe have been identified among the enteric bacteria are capable of producing histamine in fish causing histamine poisoning or Scombroid poisoning which is one of the most significant cause of illness associated with seafood [3]. It is mostly found in natural environments and is responsible for the infection of the pulmonary system, burns, skin, eyes, ears, nose, and the urinary tract, as well as gastroenteritis [4] [5]. *P. mirabilis* is the third most common cause of nosocomial infections accounting for 90% of all *Proteus* infections [6].

Human infections from *Proteus mirabilis* are known to occur after the bacteria have left the intestinal tract. In debilitated patients or those receiving intravenous infusions, they cause bacteremia, pneumonia, and localized lesions and are present in urinary tract infections. Urinary tract infections and occasionally other infections are brought on by *Proteus mirabilis*. *Proteus mirabilis* and other non-fastidious gram-negative rods are mostly encountered significant isolates in many clinical specimens in all part of the world [7].

Proteus mirabilis is among of the predominant microflora in fresh seafood including fin fish and shell fish [8]. Generally in all animal production, fishes are virtually been exposed and susceptible to microbial diseases which are the major problem hampering production, development and expansion of the aquaculture industry [9]. Fish infections are global issues that impact freshwater, marine, sport, and even ornamental fish. The problem is extremely important when fish are subjected to intensive culture practices [10]. Foodborne diseases are a widespread and growing public health concern in developed, developing and under-developed countries of the world because of the health hazards they constitute [11].

Since fish are frequently farmed in systems where output is dependent on the environment, controlling fish illnesses is particularly challenging. Changes or deterioration in the aquatic environment cause the occurrence of most fish disease and also environmental effects play a great role in influencing the health status of fish [12]. Therefore, the multidisciplinary approaches involving the characteristics of potential pathogenic microorganisms for fish, aspects of the

biology of fish as well as a better understanding of the environmental factors affecting such cultures will allow the application of adequate measures to prevent and control the diseases limiting fish production [10] [13].

Antibiotic resistance is a significant global health issue that has a negative influence on patient costs, morbidity, mortality, and duration of hospital stays [14] [15]. Numerous variables, including selection pressure brought on by the use and abuse of antibiotics in veterinary medicine, human medicine, agriculture, and other fields, have been implicated in the evolution of antibiotic resistance. Microorganisms that are periodically linked to several disease outbreaks around the world have developed multi-drug resistance as a result of the overuse and abuse of antibiotics in both clinical and community settings [16] [17] [18]. Antibiotic resistance has increased as a result of the lack of routine evaluations of the effectiveness and potency of the numerous brands of antimicrobial medicines accessible on the pharmaceutical markets, particularly in poor nations [18]. Drugs could enter the country illegally through the back door in the majority of underdeveloped nations, including Nigeria, without being examined by the appropriate regulatory organizations. Due to their desire to get wealthy overnight, some disloyal people have been adulterating and counterfeiting medicines, notably antibiotics, which has had a significant negative impact on the emergence of antibiotic resistance. Consuming partially cooked fish meals, buying roasted fish from street vendors, handling fish by hand, and unclean filleting practices in Nigeria all point to a heightened risk of infection for the general public [19]. Due to its effects on the fishing business as well as its potential threat to the aquaculture industry and public health, the bacteria deserve to study. Virtually not much study has been done in isolating the organism from fish in Nigeria. Hence our study will aim at determining the phenotypic characters of *P. mirabilis* isolated using Microbact^{TM}24E system and antimicrobial susceptibility from fresh water fishes in Abuja metropolis.

2. Materials and Methods

2.1. Study Design

The study is designed to be a cross-sectional study, also known as an observational study, includes purchase of samples from different fish markets within the FCT and determining whether or not each sample simultaneously has the agents or not as well as the anticipated risk factor. The fish were purchased from the market from three designated areas from the Federal Capital Territory Abuja, Abuja municipal Area Council, Bwari Area Council and Gwagwalada Area Council respectively [20].

2.2. Sampling Technique

A purposive sampling technique was used based on the convenience and availability of fish from ponds and market in the FCT. A simple random sampling technique was employed to select fish from ponds and market from some se-

lected Local government Areas (LGA) within the FCT.

2.3. Sample Size

Sample Size Determination

Sample size will be determined using the formula as described by Thrusfield [21], using 50% prevalence 384 samples will be obtain.

$$N = Z^2 pq/d^2$$

where:

N = Sample size

Z = 1.96 (normal distribution) from table

P = Prevalence rate from the average of previous studies

D = Desired absolute precision of 5% with 95% Confidence Interval

$q = 1 - p$

$$N = \frac{(1.96)^2 \times 0.50 \times (1-0.50)}{(0.05)^2}$$

$$N = \frac{3.84 \times 0.50 \times 0.5}{0.0025}$$

$$N = \frac{3.84 \times 0.025}{0.0025}$$

$$N = \frac{0.96}{0.0025} = 384$$

But a total of 400 samples were collected to increase precision.

2.4. Sample Collection

400 fish samples were purchased conveniently from the three area councils (Bwari, Abuja Municipal, and Gwagwalada). The sample sizes were sheared across the three area council at the time of collection. The fish samples were transported aseptically in the same day in an ice-parked container to microbiology laboratory of the University of Abuja, Department of Veterinary Microbiology Faculty of Veterinary Medicine for examination. The duration of collection of sample was from August, 2021 to February, 2022.

2.5. Necropsy and Tissue Processing

After cleaning the surface with 70% alcohol, the ventral route to the kidney was used to dissect the fish. Using sterile dissecting scissors, the fish sample's abdomen was first cut along the midline from the anus up to the mouth, then again from the anus to the lateral line and further along the lateral line up to the gills cover to remove the lateral side of the abdominal wall and reveal the internal organs. The condition of the internal organs was inspected for any gross pathological lesion. The samples were collected aseptically using sterile forceps, scalpels, blades and scissors. The entire necropsy and tissue sampling process was completed in an aseptic environment [9].

2.6. Laboratory Culture and Identification

2.6.1. Isolation Proteus Mirabilis

Isolation of *Proteus mirabilis* was carried out according to the International Organization for Standardization Guideline (ISO 6579, 2017) for isolation and characterization of *Enterobactereaca*. Aseptically extracting 1g of tissue sample, which was then homogenized in 9 mL of buffered peptone water (LabM®, UK) in a test tube to create a dilution of 1:10, was used to perform non-selective pre-enrichment. Test tubes were properly corked, labeled, and kept at 37˚C overnight. A little under 0.1 mL of the pre-enrichment was incubated at 37˚C overnight on Rappaport-Vassiliadis (RV) (Oxoid®, England) for Selective enrichment. By plating 10 L onto Xylose Lysine Deoxycholate-(XLD) agar (Himedia, India), selective agar plating was carried out and incubated at 37˚C for an overnight period. Suspect colonies with primarily black surfaces and small transparent black centers were sub-cultivated onto Xylose Lysine Deoxycholate (XLD) (Himedia, India) and incubated overnight (18 - 24 hours) at 37˚C for sub-cultivation/purification. Pure cultures were then kept on nutrient agar slants and stored at 2˚C to 8˚C in the refrigerator after being incubated at 37˚C overnight [19].

2.6.2. Primary Identification of Isolates

Using gram staining, oxidase test, and a catalase test, preliminary screening and identification were carried out. For additional biochemical characterization, Gram negative, oxidase, and catalase positive isolates were kept on nutrient agar slant.

2.7. Complete Biochemical Identification

Biochemical test such as triple sugar iron (TSI), indole productin, citrate utilization, voges proskauer, methyl red and urease test.

2.8. Microbact 24E Identification *Proteus mirabilis*

2.8.1. Principle of the Microbact™ Test

The Microbact Gram-Negative system kit is a standardized micro-substrate system created to stimulate common biochemical substrates for the identification of *enterobacteriaceae* and other common gram-negative bacteria (MGNB). Organism identification is based on pH change and substrate utilization [22]. Two substrate strips, 12A and 12B, make up the Microbact Gram-negative product. There are 12 different biological substrates in each strip. The 12A strip can be used on its own to identify oxidase-negative, nitrate-positive glucose fermenters from 15 different genera. It can also be used to screen for pathogenic *Enterobacteriaceae* in urine and intestinal samples, as well as to identify other common isolates. The 12B strip can be used in the conjunction with the 12A strip for the identification of oxidase-positive, nitrate-negative and glucose non-fermenters (MGNB) as well as the *Enterobacteriaceae* [22].

2.8.2. Procedure for Microbact 24E GNB Identification Test Kit

A suspension of the overnight culture of the organism was emulsified in 5 ml sterile saline solution and then adjusted to 0.5 McFarland turbidity standards (approximately equal to 1.5×10^8 CFU/ml of the bacterial suspension). By severing the sealing strip's end tag and progressively peeling it backward, the wells of each unique substrate are exposed. A sterile pipette was used to inject 4 drops (or roughly 100 m) of the bacterial suspension into the MICROBACT^{TM}GNB that contained the hydrated substrates after the strip was set in a holding tray. Different colored reactions (yellow, red, tan, green, and blue) are depicted in Figure 1 below. The holding tray's underlined substrates were covered with sterile mineral oil using a dropper bottle. For wells 12A or 24E, (wells 1, 2, and 3), and for wells 12B or wells 20 and 24E, (wells 8 and 12), the specimen identification number was inscribed on the end tag with a marker pen, and the inoculation rows were then sealed with an adhesive seal. 37˚C was used to incubate the strip for 18 to 24 hours. When reading the test strip (results), the reaction was compared to the color chart to determine whether it was positive or negative. Two drops of Kovac's reagent were added to wells 8 (which is where Indole is produced). Each of the VPI and VP II reagents was applied to well 10 (the Vogues-Proskauer reagent), and the TDA reagent was put to well 12 (the tryptophan deaminase). Based on an octal coding technique that Microbact employed, the results were interpreted. Each group of 3 reaction produce a single digit of the code. Using the result obtained, the indices of the positive reactions were circled. The sum of these indices in each group of the three reactions formed shown against the organism name was the percentage share of the probability for that organism [22].

2.8.3. Antibiotic Susceptibility Test

Antimicrobial susceptibility of *P. mirabilis* isolates were tested using the disk diffusion method prescribed by Kirby-Bauer *et al.* (1966) and in accordance with the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2018). The antibiotics used were Ceftriaxone (CTR, 30 µg), Gentamicin (GEN, 10 µg), Co-trimoxazole (COT, 25 µg), Levofloxacin (LE, 5 µg), Netillin (NET, 30 µg), Tetracycline (TE, 30 µg), Amoxiclav (30 µg), Ofloxacin (OF, 5 µg), Ceftazidime (CAZ, 30 µg), Erythromycin (15 µg) (Hi-media, India). An overnight culture of each isolate was prepared in nutrient broth and incubated at 37˚C for 18 h. The turbidity of the broth was adjusted to McFarland standard of 0.5. The inoculum was then spread on already prepared plates of Mueller Hinton's agar (Oxoid, UK) and left standing for 1 - 2 minutes. Using forcep, antibiotics multi-discs (Hi media, India) were aseptically placed on the inoculated plates and then incubated at 37˚C for 24 h. After incubation, the zones of inhibition were measured to the nearest millimeter using a transparent ruler and the values were recorded and interpreted as sensitive, intermediate and resistant according to CLSI, 2018 guidelines.

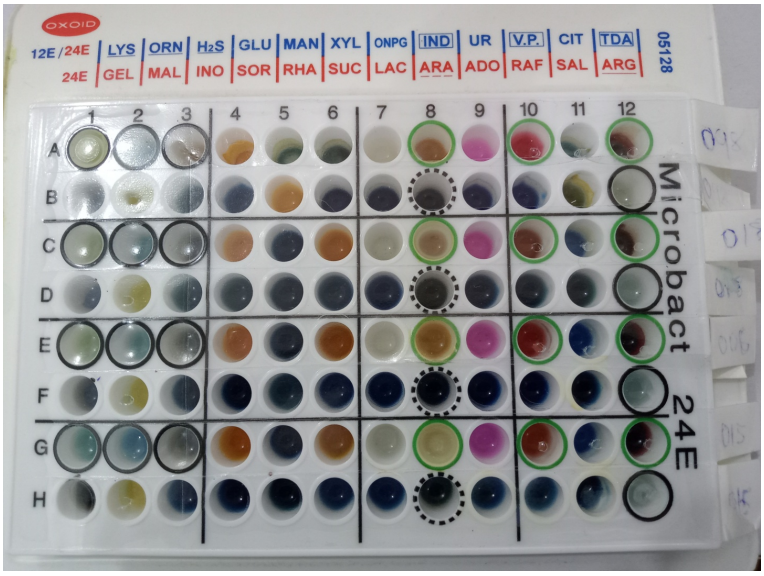

Figure 1. Pictorial presentation of Microbact™ identifying *P. mirabils* isolates after inoculation of the organism.

2.9. Data Analysis

All data were subjected to simple descriptive statistics and the results were expressed and analyzed by percentages, ratios and use of charts and tables.

3. Results

Table 1 shows the prevalence of *Proteus mirabilis* in different area councils of the FCT, Abuja. The prevalence of *Proteus mirablis* from fishes purchased in AMAC was highest at (4.81%) when compared to the prevalence of *Proteus mirabilis* from fishes purchased in Bwari (2.99%) and Gwagwalada had the prevalence of (2.57%). The overall prevalence of *Proteus mirabilis* isolates from the 400 fishes purchased in FCT was therefore 3.25%.

Table 2 shows dissipation of biochemical characterization of 13 (3.25%) isolates suspected to be *Proteus mirabilis* out of 400 fish samples used based on morphology, gram staining and complete biochemical test. All the isolates were gram negative, clear swarming growth, moist, raised and black center to colonies on Xylose Lysine Deoxycholate (XLD). All the isolates were positive for oxidase, catalase, hydrogen sulphide production, methyl red and were all motile. 13 (3.25%) isolates are positive for indole production and all isolates utilizes citrate as their source of carbon by changing the colour from green to blue. The isolates are positive for urease showing pink colouration and all 13 (3.25%) of the isolates shows positive characteristic of *Proteus mirabilis* in TSI.

Table 3 shows results of the 13 (3.25%) isolates confirmed by Microbact™GNB24E to be *Proteus mirabilis*. After subjecting the 98 suspected isolates of *Proteus*, Microbact™GNB24E confirmed 13 (3.25%) isolates to be *Proteus mirabilis.* The isolates gave various reaction as follows. All the 13 (3.25%) isolates were positive for motility, nitrate, lysine, glucose, urease, citrate, TDA, ge-

latin and arginine. 10 of the isolates reacted to xylose, 11 (2.75%) were positive to veges proskuear and 2 (2.5%) for salicin. The 13 (3.25%) isolates were negative to mannitol, ONPG, indole, Malonate, Inositol, sorbitol, sucrose, lactose, arabinose, adonitol and raffinose.

Table 4 shows the percentage distribution of *Proteus mirabilis* among fishes purchased in Bwari. The highest isolation rate is for *Lestes noliticus* (11.11%) followed by *Tilapia zillii* (2.67%), *Clarias garipinus* has (2.5%) and *Alestes nurse* has (0.00%) respectively. The total isolation rate of *Proteus mirabilis* Bwari is to be 2.99%.

Table 5 shows the percentage distribution of *Proteus mirabilis* among fishes purchased from AMAC. *Lestes noliticus* had the highest isolation rate (8.33%), followed by *Tilapia zillii* (7.50%) and then *Clarias garipinus* (2.70%). No isolation rate was recorded for *Alestes nurse* making the total isolation rate of *Proteus mirabilis* AMAC to be 4.81%.

Table 1. Prevalence of *P. mirabilis* in different area councils of Abuja.

Area councils	Number of fishes used	Number of isolate	% prevalence
AMAC	104	5	4.81%
Bwari	134	4	2.99%
Gwagwalada	162	4	2.57%
Total	400	13	3.25%

Table 2. Biochemical reactions of the suspected isolates of *P. mirabilis*.

Isolates	GS	O	C	M	TSI	VP	MR	CI	I	U
GR18	−ve	−ve	+ve	+ve	K/A + gas + H_2S	−ve	+ve	Blue	−ve	Pink
215	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
018	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
118	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
006	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
015	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
GR4	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
398	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
GR16	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
306	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
288	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
108	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink
098	−ve	−ve	+ve	+ve	++	−ve	+ve	Blue	−ve	Pink

KEY: GS = Gram staining, O = Oxidase, M = Motility, TSI = Triple Sugar Iron, VP = Voges Proskauer, MR = Methyl Red, CI = Citrate, I = Indole, U = Urease, −ve = Negative, +ve = Positive.

Table 3. Confirmation of 13 isolates of *P. mirabilis* using Microbact™GNB24E Test kit.

Biochemical	positive	Negative
Oxidase	13	0
Motility	13	0
Nitrate	13	0
Lysine	13	0
Ornithine	13	0
H_2S	11	2
Glucose	13	0
Mannitol	0	13
Xylose	10	3
ONPG	0	13
Indole	0	13
Urease	13	0
V-P	11	2
Citrate	13	0
TDA	13	0
Gelatin	13	0
Malonate	0	13
Inositol	0	13
Sorbitol	0	13
Rhamnose	2	11
Sucrose	0	13
Lactose	0	13
Arabinose	0	13
Adonitol	0	13
Raffinose	0	13
Salicin	2	11
Arginine	13	0

Table 4. Percentage distribution of *P. mirabilis* among fishes in Bwari.

Types of fishes	Number of fishes	Number of isolates	Prevalence rate %
Clarias garipinus	40	1	2.5%
Tilapia zillii	75	2	2.67%
Lestes noliticus	9	1	11.11%
Alestes nurse	10	0	0.00%
Total	134	4	2.99%

Table 6 shows the percentage of distribution of *Proteus mirabilis* among fishes purchased from Gwagwalada. *Clarias garipinus* had the highest isolation rate

(4.44%) and *Tilapia zillii* (2.04%). No isolation rate was recorded for *Lestes noliticus* and *Alestes nurse* respectively. The overall total isolation rate of *Proteus mirabilis* from fishes in Gwagwalada is to be 2.47%.

Table 7 shows the number of *Proteus mirabilis* were resistant, intermediate and sensitive to antimicrobial agent used using CLSI, 2020 break point (≥20 susceptible, 15 - 19 intermediate and ≤14 resistant). 100% of the isolates are susceptible to Ofloxacin, 922.3% were susceptible to Levofloxacin and Netillin while 7.7% were intermediate for Netillin. 76.9% were susceptible to Ceftazidime, while Co-trimoxazole (69.2%), Gentamicin (61.5%) and Ceftriaxone (30.8%) rate of susceptibility. There was 0% of susceptibility from Tetracycline, Amoxyclav, and Erythromycin. Amoxyclav shows the highest form of resistance of 100% followed by Tetracycline and Erythromycin (92.3%) each.

Table 5. Percentage distribution of *P. mirabilis* among fishes in AMAC.

Types of fishes	Number of fishes	Number of isolates	Prevalence rate %
Clarias garipinus	37	1	2.70%
Tilapia zillii	40	3	7.50%
Lestes noliticus	12	1	8.33%
Alestes nurse	15	0	0.00%
Total	104	5	4.81%

Table 6. Percentage distribution of *P. mirabilis* amongst fishes in Gwagwalada metropolis.

Types of fishes	Number of fishes	Number of isolates	Prevalence rate %
Clarias garipinus	45	2	4.44%
Tilapia zillii	98	2	2.04%
Lestes noliticus	9	0	0.00%
Alestes nurse	10	0	0.00%
Total	162	4	2.47%

Table 7. Antibacterial susceptibility of the isolates of *P. mirabilis* to different antibacterial agents.

Antibacterial agents	Sensitivity	Intermediate	Resistance
Ceftriaxone	4 (30.8%)	6 (46.2%)	3 (23.1%)
Gentamicin	8 (61.5%)	3 (23.1%)	2 (15.4%)
Co-trimoxazole	9 (69.2%)	0 (0%)	4 (30.8%)
Levofloxacin	12 (92.3%)	0 (0%)	1 (7.7%)
Netillin	12 (92.3%)	1 (7.7%)	0 (0%)
Tetracycline	0 (0%)	1 (7.7%)	12 (92.3%)
Amoxyclav	0 (0%)	0 (0%)	13 (100%)
Ofloxacin	13 (100%)	0 (0%)	0 (0%)
Ceftazidime	10 (76.9%)	2 (15.4%)	3 (23.1%)
Erythromycin	0 (0%)	1 (7.7%)	12 (92.3%)

4. Discussions

Proteus mirabilis are capable of growing on the majority of popular culture media used in clinical laboratories, including Xylose Lysine Deoxycholate (XLD) agar, nutritional agar, and sheep blood agar (SBA). The bacteria are known to have swarming characteristics with putrefactive (fishy odour) on nutrient agar which is indicated in our findings [2]. The colonies were found to be moist, small and transparent with black center and predominantly black colonies depicting hydrogen sulphide production on XLD in this study [11]. The organism can also grow in common culture media, such as MacConkey Agar and Cystein Lactose Electrolyte Deficient (CLED) media, used for the isolation of enteric bacteria. The isolation of the *Proteus mirabilis* from four different species of commonly consumed fish in this study area gives insight to fish being the possible sources of transmission of *Proteus* species infection via the food chain processed from the farmers, fishermen and down to the end user consumer. A medium containing heart infusion agar supplemented with bile salts, lithium chloride, sodium thiosulfate and sodium citrate was developed for selective growth of *proteus* [2].

About 400 fish samples were investigated in this research work. Peptone water was used for pre-enrichment, and Rappaport-Visilliadis broth was used for selective enrichment. It was then cultured into Xylose Lysine Deoxycholate (XLD) agar because according to [9] the said media are widely used for the isolation of *Proteus* species from conterminated materials. In our study, we isolated *Proteus mirabilis* from fresh water fish using XLD media, which grow at 37˚C. All isolates shows swarming characteristics with a fishy odour, transparent with black center to predominantly black colonies, this finding implies that fresh water fish in the FCT are heavily contaminated with the bacteria.

A number of biochemical tests, including those for catalase, oxidase, citrate utilization, urease, methyl red, voges proskauer, motility, indole, triple sugar iron, and sugar fermentation, such as the xylose and glucose tests, revealed quantitative phenotypic reactions typical of *Proteus mirabilis* and can reveal the dominant species of the organism in a particular geographic area. The test with the highest positive predictive value included 98 isolates, all of which were gram negative, oxidase negative, catalase positive, indole negative, motile, citrate positive, glucose and xylose positive and few negative, urea positive and few negative, and suspected to be other Proteus species. Biochemical tests therefore are significant tool in the understanding the prevailing biotypes and species of *Proteus* species prevalent in the area covered by our study. Further other biochemical test was necessary to reveal the predominant species in FCT, Nigeria.

From our study, 13 isolates were suspected to be *Proteus* species going by the conventional biochemical test conducted. Owoseni *et al.*, [11], found *Proteus* species to be 74 (34.3%) of the samples from poultry farms in Lafia, Nasarawa state, Nigeria through conventional biochemical studies which is at variance with our studies. As an alternative to biochemical procedures, it is also necessary

to investigate innovative techniques for *Proteus* identification. This may be because biochemical procedures can be time-consuming and have a potential of producing false-positive results. In spite of all the odds, biochemical tests continue to be one of the most effective ways to identify *Proteus* strains and other related organisms from tainted materials. Additionally, biochemical tests might be used as a first step before applying quick test techniques to confirm field isolates. It may be possible to identify new Proteus species in Nigeria by using scientific information and expertise for recognizing common Proteus species in fish.

Proteus mirabilis utilizing the Microbact™24E GND kit (Oxoid, England), a commercial biochemical kit in micro plate format for identifying *Enterobacteriaceae* and gram negative bacilli, additional analytical profile index testing was performed on the organisms identified by the conventional biochemical test reaction. Organisms' identification is based on pH change and substrate utililisation as established by published referenced methodologies [22]. The kit features a computer-aided identification software called Microbact™ that is used to identify items from a list using a series of numbers. The probability for that organism as a proportion of the total probabilities for all options is represented by the percentage value next to the organism name. From the Microbact 24E identification, 13 isolates were identified as *Proteus mirabilis*. It has been used by different researchers such Tamba *et al.*, [23]; Adeyemi and Akinde, [24] for the identification of *Proteus* species. It is cheaper, easier, more convenient and effective to use than the conventional biochemical methods. This is attributed to its simple and automated nature in the identification of the individual microorganisms from various samples within the environment.

According to Tamba *et al.*, [23], identification of *Proteus mirabilis* to genus level can be conducted using routine test employed in the identification of other enteric bacteria. Commercial kit such as Microbact 24E could be used in the confirmation of *Proteus mirabilis* and have been able to identify more than 95% of *Proteus* to genus level when compared with data obtained from molecular diagnosis [24]. The prevalence rate of 3.25% of *Proteus mirabilis* in fish in the 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). The isolation method used, such as the type of media use, season of the year as well as location of isolation. Umar *et al.*, [25], had a prevalence of 70% from urinary tract infection patient attending sickbay Hospital in Zaria; Owoseni *et al.*, [11] had prevalence of 34.3 from chicken dropping and drinking water of poultry farms in Lafia.

The overall prevalence rate of *Proteus mirabilis* isolated from different fresh water fishes purchased from different markets within the FCT is 3.25% is lower than the prevalence rate found by Owoseni *et al.*, [11], Qasim, [3] and Gufe *et al.,* [10] with prevalence rate of (34.3%), (39.16%) and (5%) each respectively. This may be due to the type and size of samples used by the different research-

ers, which Owoseni *et al.* [11] use chicken droppings and drinking water from poultry farms. This finding was also less than the findings of Umar *et al.*, [25] which had prevalence of 20% from urine sample of patients suspected with urinary tract infections attending sickbay. This prevalence is possible because *Proteus* has been associated with urinary tract infections in several reports. Zafar *et al.*, [6] also reported a higher prevalence of (13.3%) from patients' wounds attending Hospital. The prevalence of *P. mirabilis* from fishes purchased in AMAC was highest at (4.81%) when compared to the prevalence of *P. mirabilis* from fishes purchased in Bwari (2.99%) and Gwagwalada with prevalence rate of (2.47%).

P. mirabilis was the only specie of the genus *Proteus* that was isolated in this study, which agrees with the findings of Gufe *et al.*, [10] who reported that the isolation and identification of *P. mirabilis* and other enteric bacteria species in fish indicate multisource pollution of fish from sewage effluents, humans during handling, industrial and agricultural wastes [10]. *P. mirabilis* is also a commensal in warm blooded animals; therefore, its presence might indicate to feacal contamination of the water of any environment that it is found. The organism is an opportunistic infection that mainly affects people with compromised immune systems.

The distribution of *P. mirabilis* in Federal Capital Territory showed that *Lestes noliticus* had the highest prevalence of *P. mirabilis* (11.11%) in Bwari while in AMAC (8.33%) and *Clarias garipinus* had the highest prevalence in Gwagwalada (4.44%). All the fish species showed varying prevalence in different fishes and different areas of the FCT. This is in agreement with previous work of Mailafia *et al.*, [26]. This clear variation in infection rates in different under studied areas. There was no any prevalence rate recorded for *Alestes nurse* in all the area councils. This may be attributed to small sample size, system of production, season of the year, culture methods or other geographical factors. *P. mirabilis* has been associated in human infection; the prevalence in fish may be potential sources of human infection especially where fish and fish products are not properly handled before consumption [27].

All *P. mirabilis* isolates encountered were 100% resistance to Amoxicillin and 92.3% were resistance to Erythromicin, this similar to the findings of Zafar *et al.*, [6] who stated that *P. mirabilis* shows 93.7% resistance to Amoxicillin and Umar *et al.*, [25] reported 100% resistance to Amoxicillin which agree with our findings but 0% resistance of *P. mirabilis* to Erythromicin. Our findings does not agree with the study of Owoseni *et al.*, [11], who report that isolates of *P. mirabilis* show 2.7% resistance to Erythromicin. 92.3% were resistance to Tetracycline, which also corroborate with the study of Zafar *et al.*, [6], who reported 92% of *P. mirabilis* isolates show resistance to tetracycline. Multiple resistances was observed in this study and is relatively similar with other studies that have been reported by Zafar *et al.*, [6], who indicated that *P. mirabilis* in their study showed resistances to more than two antibiotic especially Erythromicin, Amox-

icillin and Tetracycline. Antibiotic handling and sales regulations should be upheld to prevent indiscriminate drug usage that could result in sub therapeutic dosage, accelerating the emergence of resistance mutants. It is strongly advised that the general public be made aware of personal hygiene for people, fish handlers, and the environment. Increased fish farming has resulted in increased in the use of antimicrobial agents for prevention and therapeutic treatment of bacterial fish diseases [27]. The extensive use of antibiotics in human and veterinary medicine, aquaculture agriculture is contributing to the selection and dissemination of antibiotic resistant microorganisms, probably by the transferring resistant plasmid integrons [27]. Regulating the use of antimicrobial drugs as growth promoters in aquaculture is crucial for reducing the spread of antimicrobials that result from aquaculture. Therefore, the presence of the bacterium in fish poses a risk to human health because the food chain may pass the resistance to humans. Plasmids are extra-chromosomal DNA ending virulence determinants [16]. *P. mirabilis* contains stable plasmids that are crucial to the virulence and resistance of microbe.

All *P. mirabilis* isolates from this study were 100% sensitive to Ofloxacin, 92.3% were sensitive to Levofloxacin and Netillin which corroborates with the findings of Fernandez-Delgado *et al.*, [28], who noted that *P. mirabilis* associated with two species of Venezuelan *Oysters* were sensitive to Netillin. 76.9% of the isolates were sensitive to Ceftazidime, which agrees with the study of Zafar *et al.*, [6], who noted that about 78.8% of *P. mirabilis* isolates from patient wounds were susceptible to Ceftazidime. Kwiecinska-Pirog *et al.*, [29], also noted from their study that 52.0% and 76% of *P. mirabilis* were from urine and wound swab and were susceptible to Ceftazidime. 69.2% were sensitive to Co-trimoxazole and 61.5% of the isolates were sensitive to Gentamicin, which concur to the findings of Mirzaei *et al.*, [30], who reported that *P. mirabilis* isolated from patients with Urinary Tract Infection (UTI) in Iran was susceptible to Co-trimoxazole and Ceftazidime. Susceptibility to Gentamicin is in agreement with study of Gufe *et al.*, [10], who reported the *P. mirabilis* isolated from fish sold at informal Market in Mufakosa, Zimbabwe is susceptible to Gentamicin. Umar *et al.*, [25] also reported in a study in Zaria, Nigeria that *P. mirabilis* isolates are susceptible to Gentamicin. 30.8% were susceptible to Ceftrixone, which corroborates with the findings of Zafar *et al.*, [6], who noted that *P. mirabilis* isolates were susceptible to Ceftrixone and Gentamicin in their study. The presence of resistance or susceptibility indicates rampat use and misuse of antimicrobial agent in the environment covered by this study. The bacteria could also acquire inherent factors from the environment that could generate resistance. There is therefore need for public health concern and government to employ stringent measures and regulations in the control of antibiotics use in fishes.

5. Conclusion

With an overall prevalence of 3.25%, this study confirmed the isolation and con-

firmation of *P. mirabilis* using both conventional biochemical methods and the Microbact™ 24E identification kit. The isolates showed multiple susceptibility to Ofloxacin (100%), Netillin (92.3%), Levofloxacin (92.3%), Ceftazidime (76.9%), Co-trimoxazole (69.2%) and Gentamicin (61.5%). The resistant antibiotics were: Amoxyclav (100%), Erythromycin (92.3%), Tetracycline (92.3%) and Ceftriaxone (23.1%). Since *P. mirabilis* has been linked to human illness, the presence of *P. mirabilis* in fish may represent a potential risk factor for human infection, particularly in situations where fish and fish products are not treated adequately before eating.

Conflicts of Interest

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

References

[1] Abed, M.K. and Shareef, H.K. (2021) Isolation and Molecular Identification of *Proteus mirabilis* Isolated from Hospitals in the Capital Baghdad. *Indian Journal of Forensic Medicine & Toxicology*, **15**, 2216-2223.

[2] Hussein, E.I., Al-Batayneh, K., Masadeh, M.M., Dahadhah, F.W., Al Zoubi, M.S., Aljabali, A.A. and Alzoubi, K.H. (2020) Assessment of Pathogenic Potential, Virulent Genes Profile, and Antibiotic Susceptibility of *Proteus mirabilis* from Urinary Tract Infection. *International Journal of Microbiology*, **2020**, Article ID: 1231807. https://doi.org/10.1155/2020/1231807

[3] Qasim, D.A. (2020) Screening the *Proteus mirabilis* by Isolation from Tuna Fish and Compared the Occurrence with Local Fish. *Biochemical and Cellular Archives*, **20**, 481-486.

[4] Al-Ezzy, A.I., Khazzal, S.A. and Qassim, A.R. (2018) Isolation of *Proteus mirabilis* from Urinary Tract Infections of Human and Ovine in Baqubah-Diyala Province. *Diyala Agricultural Sciences Journal*, **10**, 339-347.

[5] Yuan, F., Huang, Z., Yang, T., Wang, G., Li, P., Yang, B. and Li, J. (2021) Pathogenesis of *Proteus mirabilis* in Catheter-Associated Urinary Tract Infections. *Urologia Internationalis*, **105**, 354-361. https://doi.org/10.1159/000514097

[6] Zafar, U., Taj, M.K., Nawaz, I., Zafar, A. and Taj, I. (2019) Characterization of *Proteus mirabilis* Isolated from Patient Wounds at Bolan Medical Complex Hospital, Quetta. *Jundishapur Journal of Microbiology*, **12**, e87963. https://doi.org/10.5812/jjm.87963

[7] Maina, J., Ndung'u, P., Muigai, A., Onyango, H., Mukaya, J.K., Wambui, S. and Kiiru, J. (2019) Antimicrobial Profiles of Selected Gram-Negative Bacteria Recoverable from Sewage and Sludge from Juja and Kibera Informal Settlements of the Larger Nairobi Metropolis. *Advances in Microbiology*, **9**, 507-524. https://doi.org/10.4236/aim.2019.96031

[8] Singh, A.S., Lekshmi, M., Prakasan, S., Nayak, B.B. and Kumar, S. (2017) Multiple Antibiotic-Resistant, Extended Spectrum-β-Lactamase (ESBL)-Producing Enterobacteria in Fresh Seafood. *Microorganisms*, **5**, Article 53. https://doi.org/10.3390/microorganisms5030053

[9] Kebede, B. and Habtamu, T. (2016) Isolation and Identification of *Edwardsiella tarda* from Lake Zeway and Langano, Southern Oromia, Ethiopia. *Fisheries and*

Aquaculture Journal, **7**, Article ID: 1000184.
https://doi.org/10.4172/2150-3508.1000184

[10] Gufe, C., Hodobo, T.C., Mbonjani, B., Majonga, O., Marumure, J., Musari, S., Jongi, G., Makaya, V. and Machakwa, J. (2019) Antimicrobial Profiling of Bacteria Isolated from Fish Sold at Informal Market in Mufakose, Zimbabwe. *International Journal of Microbiology*, **2019**, Article ID: 8759636. https://doi.org/10.1155/2019/8759636

[11] Owoseni, M.C., Oyigye, O., Sani, B., Lamin, J. and Chere, A. (2021) Antimicrobial Resistance and Virulence Genes Profile of Proteus Species from Poultry Farms in Lafia, Nigeria. *bioRxiv*. https://doi.org/10.1101/2021.01.07.425673

[12] Noor, E.I., Deen, A.I.E., El-Gohary, M.S., Abdou, M.S., Adel, M. and Gamal, E.I. (2017) Molecular Characterization of *Edwardsiella tarda* Bacteria Causing Severe Mortalities in Cultured *Oreochromeis niloticus* Fish with Treatment Trials. *International Journal of Current Research*, **9**, 50962-50969.

[13] Nasr-Eldahan, S., Nabil-Adam, A., Shreadah, M.A., Maher, A.M. and El-Sayed Ali, T. (2021) A Review Article on Nanotechnology in Aquaculture Sustainability as a Novel Tool in Fish Disease Control. *Aquaculture International*, **29**, 1459-1480.
https://doi.org/10.1007/s10499-021-00677-7

[14] Founou, R.C., Founou, L.L. and Essack, S.Y. (2017) Clinical and Economic Impact of Antibiotic Resistance in Developing Countries: A Systematic Review and Meta-Analysis. *PLOS ONE*, **12**, e0189621.
https://doi.org/10.1371/journal.pone.0189621

[15] Shrestha, P., Cooper, B.S., Coast, J., Oppong, R., Do Thi Thuy, N., Phodha, T., Celhay, O., Guerin, P.J., Wertheim, H. and Lubell, Y. (2018) Enumerating the Economic Cost of Antimicrobial Resistance Per Antibiotic Consumed to Inform the Evaluation of Interventions Affecting Their Use. *Antimicrobial Resistance & Infection Control*, **7**, Article No. 98. https://doi.org/10.1186/s13756-018-0384-3

[16] Alabi, O.S., Mendonça, N., Adeleke, O.E. and da Silva, G.J. (2017) Molecular Screening of Antibiotic-Resistant Determinants among Multidrug-Resistant Clinical Isolates of *Proteus mirabilis* from South-West Nigeria. *African Health Sciences*, **17**, 356-365. https://doi.org/10.4314/ahs.v17i2.9

[17] Chaw, P.S., Höpner, J. and Mikolajczyk, R. (2018) The Knowledge, Attitude and Practice of Health Practitioners towards Antibiotic Prescribing and Resistance in Developing Countries—A Systematic Review. *Journal of Clinical Pharmacy and Therapeutics*, **43**, 606-613. https://doi.org/10.1111/jcpt.12730

[18] Chokshi, A., Sifri, Z., Cennimo, D. and Horng, H. (2019) Global Contributors to Antibiotic Resistance. *Journal of Global Infectious Diseases*, **11**, 36-42.
https://doi.org/10.4103/jgid.jgid_110_18

[19] Ogunleye, S.C., Ishola, O.O., Olatoye, I.O. and Adedeji, O.B. (2020) Detection and Antibiogram of *Edwardsiella tarda* from *Oreochromis niloticus* (Tilapia) Obtained from Selected Farms in Ibadan, Nigeria. *Journal of Food Safety and Hygiene*, **6**, 38-46.

[20] Etuk, M.N., Igwe, O. and Egbueri, J.C. (2022) An Integrated Geoinformatics and Hydrogeological Approach to Delineating Groundwater Potential Zones in the Complex Geological Terrain of Abuja, Nigeria. *Modeling Earth Systems and Environment*, **9**, 285-311. https://doi.org/10.1007/s40808-022-01502-7

[21] Thrusfield, M. (2005) Veterinary Epidemiology. 2nd Edition, Blackwell Scientific Publications, Oxford.

[22] Balows, A., Hustler, K.L., Herrmann, J.D., Isengerg, H. and Shadomy, J. (1991) Manual of Clinical Microbiology. 5th Edition, American Society of Microbiology,

Washington DC.

[23] Tamba, Z., Ndams, I.S., Oniye, S.J., Kwaga, J.K.P. and Wada, Y.A. (2021) Microbact 24E Detection and Prevalence of Faecal Enterobacteriaceae Isolates from Lactating Cattle in Jos-Plateau and Environs, Nigeria. *Dutse Journal of Pure and Applied Science* (*DUJOPAS*), **7**, 2635-3490.

[24] Adeyemi, F.M. and Akinde, S.B. (2021) ESβL, AmpC and Carbapenemase Co-Production in Multi-Drug Resistant Gram-Negative Bacteria from HIV-Infected Patients in Southwestern Nigeria. *African Journal of Clinical and Experimental Microbiology*, **22**, 38-51. https://doi.org/10.4314/ajcem.v22i1.6

[25] Umar, M., Yaya, A.A., Yusuf, G., Tafinta, I.Y., Aliko, A.A., Jobbi, D.Y. and Lawal, G. (2016) Biochemical Characterization and Antimicrobial Susceptibility Trends of *Proteus mirabilis* Isolated from Patients Suspected with Urinary Tract Infections Attending Sickbay Hospital, Zaria, Kaduna, Nigeria. *Annals of Biological Sciences*, **4**, 1-8.

[26] Mailafia, S., Nabilah, B. and Olabode, H.O.K. (2021) Phenotypic Characterization of *Aeromonas hydrophilia* Isolates in Fresh Water Fishes in FCT Using Microbact™ GNB 24E Identification Kit. *Open Access Library Journal*, **8**, 1-2.

[27] Effiong, M.U. and Christopher, E.U. (2020) Public Health Implication of Microbial Loads in Smoked Mackerel, *Scomber scombrus* from Major Fish Markets in Uyo, Akwa Ibom State, Nigeria. *South Asian Research Journal of Agriculture and Fisheries*, **2**, 126-131.

[28] Fernandez-Delgado, M., Contreras, M., Garcia-Amado, M.A., Gueneau, P. and Suarez, P. (2007) Occurrence of *Proteus mirabilis* Associated with Two Species of Ve nezuelan Oysters. *Revista do Instituto de Medicina Tropical de São Paulo*, **49**, 355-359. https://doi.org/10.1590/S0036-46652007000600004

[29] KwieciNska-Piróg, J., Skowron, K., Zniszczol, K. and Gospodarek, E. (2013) The Assessment of *Proteus mirabilis* Susceptibility to Ceftazidime and Ciprofloxacin and the Impact of These Antibiotics at Subinhibitory Concentrations on *Proteus mirabilis* Biofilms. *Biomedical Research International*, **2013**, Article ID: 930876. https://doi.org/10.1155/2013/930876

[30] Mirzaei, A., Habibi, M., Bouzari, S. and Karam, M.R.A. (2019) Characterization of Antibiotic-Susceptibility Patterns, Virulence Factor Profiles and Clonal Relatedness in *Proteus mirabilis* Isolates from Patients with Urinary Tract Infection in Iran. *Infection and Drug Resistance*, **12**, 3967-3979. https://doi.org/10.2147/IDR.S230303