



## Detection and antibiogram of *Edwardsiella tarda* from *Oreochromis niloticus* (Tilapia fish) obtained from selected farms in Ibadan, Nigeria

Ogunleye Seto Charles\*, Ishola Olayinka Olabisi, Olatoye Isaac Olufemi, Adedeji Olufemi Bolarinwa

<sup>1</sup>Department of Veterinary Public Health and Preventive Medicine University of Ibadan, Nigeria.

### ARTICLE INFO

#### Article history:

Received 09 Jan. 2020  
Received in revised form  
21 Mar. 2020  
Accepted 27 Mar. 2020

#### Keywords:

Antibiogram,  
*Edwardsiella tarda*,  
*Oreochromis niloticus*

### ABSTRACT

*Edwardsiellosis* caused by *Edwardsiella tarda* is a septicemic bacterial disease responsible for 5-70% mortalities and prevalence up-to 70% in freshwater fishes. Although rarely associated with human infections, *Edwardsiella tarda* have been found to cause gastroenteritis, soft tissue infection, liver abscess, tubo-ovarian abscess, and mycotic aneurysm mostly in immunocompromised humans. This study investigated prevalence and antibiogram of *E. tarda* isolated from *Oreochromis niloticus* obtained from selected farms in Ibadan, Nigeria. A total of 156 samples consisting: gills, intestines and skins were collected from 52 *O. niloticus* from Egbeda-(A), Ido-(B), Ibadan: North-East-(C) and North-West-(D) for bacteriological analysis. *E. tarda* Isolation, identification, and antibiogram were performed using standard methods. Data were analyzed using Chi-Square. An overall prevalence of 62.5% was observed for *E. tarda* with 87.5%, 62.5% and 50.0% for gills, intestine and skin samples, respectively, whilst overall location prevalence were observed as: 100.0%-(A), 50.0%-(B), 66.6%-(C) and 50.0%-(D). Isolates exhibited resistance patterns comprising; 100.0%-(Ceftazidime-(CPZ), Cefuroxime-(CRX) and Meropenem-(MEM)), 91.7%-Cefotaxime-(CTX), 83.3%-(Tetracyclin-(TET)), 50.0%-(Cotrimoxazole-(COT)), 33.3% (Ceftriaxone-(CTR) and Gentamicin-(GEN)), 25.0%-(Chloramphenicol-(CHL)), 16.7%-Amikacin-(AMK) and 8.3%-(Ciprofloxacin-(CIP)). Multi-drug resistance pattern: CRX-CFZ-MEM-(100%), CRX-CTR-CTX-CFZ-MEM-(83.3%), CRX-CTR-CTX-CFZ-MEM-TET-(66.7%), CRX-CTR-CTX-CFZ-MEM-TET-COT-(58.3%) and CRX-CTR-CTX-CFZ-MEM-TET-COT-GEN-(8.3%) was observed. Isolation and identification of *E. tarda* from *O. niloticus* confirm its presence in Ibadan and affirms that *O. niloticus* harbors, and could serve as a source of infection to humans. The antimicrobial resistance patterns of isolates to antibiotics indicate misuse in aquaculture and indiscriminate disposal of antibiotics into aquatic environments. This suggests risks of transmission of infectious agents to human and probable spread of resistant pathogens to humans from the environment.

**Citation:** Charles O S, Olabisi IO, Olufemi OI, Bolarinwa AO. **Detection and antibiogram of *Edwardsiella tarda* from *Oreochromis niloticus* (Tilapia fish) obtained from selected farms in Ibadan, Nigeria** . J food safe & hyg 2020; 6(1): 38-46.

### 1. Introduction

The persistent increase in global human population with its consequential increase in demands for food of animal origin has contributed to the increasing demands for the aquaculture products. FAO, 2018 (1)

reported in its annual report that “aquaculture products have become the fastest growing source of protein worldwide”, this, evidencing in the rapid increase in global aquaculture practices so as to meet the human demands for foods of animal origin. As part of the global concentration in aquaculture, *Oreochromis niloticus* commonly known as Tilapia has been known to be the most cultured fish around the world (3).

\*Corresponding author. Tel.: +2348068029003  
E-mail address: [ogunleyeseto@yahoo.com](mailto:ogunleyeseto@yahoo.com)



As with the other developing nations', Nigeria; currently falling short the National demands for aquatic products have recently increased its intensities into production of fishes. Currently, production and consumption of fish in Nigeria has become a major source of animal protein which has competed satisfactorily with meat. Fish is widely accepted in most parts of the country because of its unique taste, flavor and good texture. As part of human diet, it serves as an excellent source of high digestible proteins (4).

Surprisingly, the increasing production of aquatic products has been impacted by the ravaging effects of infectious diseases. This can be partly due to an increasing efforts channelled onto the production alone, leaving the infectious' diseases managements out of many production systems. A neglect to the infectious' diseases management have resulted in increasing economic loses, and importantly, smooth transmission of infectious diseases from humans to fish and vice versa (Zoonoses). Fish, despite several health benefits, are known to be as a source of food-borne toxoinfections to human; like other food animals (5) through the consumption of contaminated fish and fish products. Principally, the microbial flora associated with freshly harvested fish is principally function of the environment in which they are caught and not necessarily of the fish species; hence, the indigenous microbial populations of fish can vary significantly (6). Because of their soft tissues and the aquatic environment, they are extremely susceptible to microbial contamination. Bacteria, many of which are potential spoilers are present in the surface slime, gills and intestines of live fish, although the flesh itself is normally sterile (7).

*Edwardsiella tarda* associated with fishes but rarely with human infections have been recently associated with number of human infections. Hence, *Edwardsiella tarda* is specifically important for its zoonotic potentials (8, 9, 10). *Edwardsiella tarda* is a member of the *Enterobacteriaceae*, a motile, facultatively anaerobic and gram negative rod as first described in mid 1960s by Trabulsi et al in 1962 (11). Principally, *E. tarda* have been reportedly isolated from different aquatic water environments and, from the intestines of infected humans, usually following consumptions of contaminated marine and freshwater fishes such as catfish (12) or eels (13) and from other animals,

including reptiles (14). *E. tarda* is often responsible for septicemic diseases causing mass mortality in fishes and consequently, high economic losses in fish farms in many countries; causing 5%-70% mortalities (15,16, 17) and prevalence as high as 70% (9,10,18,19). *E. tarda* rarely have been associated to cause human infections, with colonization rate ranging from 0.0073% (in Japanese) (8,9) to 1% (in Panamanians) (8,10,20). In cases of human infections, approximately 80% of *E. tarda* infections in humans are characterized as gastroenteritis and extraintestinal infections which are characterised by biliary tract infection, bacteremia, skin and soft tissue infection, liver abscess, peritonitis, intra-abdominal abscess, tubo-ovarian abscess, and mycotic aneurysm mostly in immunocompromised individuals (8,10,21,22).

Prevalence of *E. tarda* in an environment has been found to be enhanced by high temperature, poor water quality and high organic content (10) and environmental associated risk factors such as; history of consumption of animals or tissues with dietary risk factors and/or mortality associated to *E. tarda* (8).

Responding to various degrees of economic losses due to infectious diseases as well as preventing zoonoses, antimicrobial agents have been devised to combat the challenges emanating from microorganisms in aquaculture practices. The inappropriate usage of these agents without adequate prescription by farmers has contributed to the current global public health concerns associated with the antimicrobial resistance (AMR). *Edwardsiella tarda*, like many other bacteria have been reported to have overtime developed resistance to several commonly used antibiotics (10,23), and drug resistant infections have been predicted to likely be associated with the mortality of up to 10 million people annually, if the current trends of antimicrobial resistance persist (24,25). Overuse of antibiotics has been accounted as the major cause of resistance (26). Antimicrobial resistance strains have evolved to become a serious health problem worldwide (27). *E. tarda* have been found show resistance to antibiotics such as; Ceftazidime, Meropenem, Cefuroxime, tetracyclin, and cotrimoxazole (10).

The radar of infectious disease epidemiology have revealed a dearth of knowledge and report about infections and public health importance of *E. tarda* in Nigeria, hence, this study was aimed at detecting, and determining the prevalence and antibiogram of *Edwardsiella tarda* from *O. niloticus* sold in Ibadan, Oyo

State. The outcomes from this study would therefore be vital tools for addressing *E. tarda* infections and zoonoses, as well as the AMR global menace, and for devising suitable preventive and control strategies in aquatic health management and public health.

## 2. Materials and Methods

### 2.1. Sampling method and sample collection

This cross-sectional study involved fish farms raising Tilapia in four Local Government Area (LGA) of Ibadan, Oyo State, Nigeria. Egbeda (A), Ibadan North West (B), Ido (C), Ibadan North East (D) LGAs in Ibadan were purposively selected based on the availability of fish farms cultivating *Oreochromis niloticus*. Four farms comprising two feral (1 and 2) from A and B LGAs and two cultured farms (3 and 4) from C and D LGAs were purposively selected for sample collection. A total of fifty two apparently healthy, table-sized (average weight 120 g) live tilapia comprising 16 each from the feral farms (1 and 2) and 10 each from cultured farms (3 and 4) were collected between July and August 2019. Sampled tilapia were hygienically and safely shipped to the Food and Milk Hygiene Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for bacteriological analysis. On arrival at the laboratory, fish were stored briefly and were properly identified and organs of interest were aseptically harvested using scalpel. One gram each of samples of gills, intestine and skin were collected and aseptically macerated separately with buffered peptone water (LabM®, UK). A total of 156 samples consisting of gills (n=52), intestines (n=52) and skins (n=52) was therefore obtained from the 52 *Oreochromis niloticus* for bacteriological analysis. Samples were processed according to the protocols recommended by microbiological Standards and Guidelines by International Organization for Standardization (ISO 6579, 2017) and National Committee for Clinical Laboratory Standards (NCCLS, 2003).

### 2.2. Isolation and identification of *Edwardsiella tarda*

Isolation of *Edwardsiella tarda* was carried out according to the International Organization for Standardization Guideline (ISO 6579, 2017) for isolation and characterisation of Enterobacteraea. Non-selective pre-enrichment was performed by aseptically harvesting 1g of tissue sample, and then homogenized in 9 mL buffered peptone water (LabM®, UK) in a test-tube to give a dilution of 1:10. Test-tubes were corked properly, labeled and incubated overnight at 37°C. About 0.1 mL of the pre-enrichment inoculated on Rappaport-Vassiliadis (RV) (Oxoid®, England) for Selective enrichment and incubated overnight at 37°C. Selective agar plating was performed by plating 10 µL onto Xylose Lysine Deoxycholate-(XLD) agar (Oxoid®), and incubated at 37°C overnight. Suspected colonies which show small transparent black centers to predominantly black colonies (fig 1) were sub-cultivated unto Xylose Lysine Deoxycholate-(XLD) (Oxoid®) and incubated overnight (18–24 hours) at 37°C for Sub-cultivation/purification. This was followed by storage of pure cultures onto nutrient agar slants, incubated at 37°C overnight and stored in the fridge at 2°C-8°C.

### 2.3. Morphological and biochemical tests

Morphological characteristics of isolates were performed through Gram staining. Biochemical characterization of isolates was performed by using Sugar fermentation tests, Catalase, Indole and TSI. Incubate at 37°C overnight.

### 2.4. Antibiotic susceptibility test

The antimicrobial susceptibility test was carried out using the agar disk diffusion method as described by Bauer *et al.* (1966) and Clinical and Laboratory Standards Institute (CLSI) 2017 was used. Antibiotic sensitivity pattern of isolated *E. tarda* was performed against 12 commonly used antibiotics belonging to different groups using commercially available antibiotic discs (Biomark Lab®) containing antibiotics at different micrograms. Amikacin (AMK, 30 µg), Cefotaxime (CTX, 30 µg), Ceftazidime (CPZ, 30 µg), Ceftriaxone (CTR, 30 µg), Cefuroxime (CRX, 30 µg), Chloramphenicol (CHL, 10 µg), Ciprofloxacin (CIP, 5 µg), Cotrimoxazole (COT, 25 µg), Gentamicin (GEN, 10 µg), Meropenem (MEM, 10 µg), Tetracycline (TET, 10

µg), Vancomycin (VAN, 30 µg) were used to determine sensitivity and resistance patterns.

After 24 h of incubation, each plate was examined. Diameters of the zones of inhibition were measured to the nearest millimetre, using ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background and zones are measured in millimetre (mm) from the upper surface of the agar illuminated with reflected light, with the cover removed (EUCAST, 2015).

## 2.5. Data analysis

Data were analyzed using descriptive statistics and Chi-Square to test association between variables.

## 2.6. Ethical approval

The study was approved by the Animal Care and Use Research Ethics (ACUREC), University of Ibadan, Nigeria with approval number: UI-ACUREC/19/0079. Samples included in this study were collected from only consenting farmers raising tilapia fish.

## 3. Results

### 3.1. Identification of *Edwardsiella tarda*

#### 3.1.1. Morphological characteristics

Isolation and identification of *Edwardsiella tarda* (*E. tarda*) through culture on Xylose Lysine Deoxycholate (XLD) Agar revealed colonies of varying sizes, small and transparent with black centers to predominantly black colonies (fig 1); microscopically, identification of isolates revealed Gram-negative short bacilli by Gram stain.

Conventional biochemical tests showed that isolates was able to produce indole and were positive in hydrogen sulphide production test (indicative of sulfite reaction by the production of black FeS) with, glucose fermentation; non-fermentable to lactose and/ or sucrose and catalase positive. From the one-hundred and fifty six (156) samples consisting 52 each of gills, intestines and skins collected from the 52 *Oreochromis niloticus*, *E. tarda* was isolated from a total of One Hundred and Five (105) samples. This represents an

overall prevalence of 62.5%. The isolates comprised of Forty-six (46) Gills, Thirty-three (33) intestines and Twenty-Six (26) skin representing 87.5%, 62.5% and 50.0%, respectively. The isolates based on location comprised of 48(A), 24(B), 20(C), and 15(D). This making the overall location prevalence of: 100.0%, 50.0%, 66.6% and 50.0% for locations A, B, C and D, respectively.

Based on the locations, prevalence; 100.0%, 100.0% and 100.0% were observed for the Gills, intestines and Skin, respectively from the Location A (n=48: Gills -16; intestines-16 and skin-16); 100.0%, 50.0% and 0.0% were observed for the Gills, intestines and Skin, respectively from the Location B (n=24: Gills -16; intestines- 8 and skin-0); 0.0%, 100.0% and 50.0% were observed for the Gills, intestines and Skin, respectively from the Location C (n=15: Gills -0; intestines-10 and skin-5); and 50.0%, 50.0% and 50.0% were observed for the Gills, intestines and Skin, respectively from the Location D (n=15: Gills -5; intestines-5 and skin-5).

A significantly association ( $p < 0.05$ ) was observed between organ types and organs' positivity (prevalence). Furthermore, A significantly association ( $p < 0.05$ ) was observed between sample locations and sample positivity (prevalence).

### 3.2. Antibigram of *Edwardsiella tarda*

Several antibiotics from different families were tested and the profiles of isolates were determined. The different diameters obtained were compared with the standards (CLSI, EUCAST) to determine whether the strains were resistant, intermediate or sensitive to each of the antibiotics. *Edwardsiella tarda* isolates showed highest resistance (100%) to Ceftazidime (CPZ), Cefuroxime (CRX) and Meropenem (MEM) and highest susceptibility to Ciprofloxacin (91.7%) (Table 1). Different levels of Multi-Drug Resistance (MDR) patterns were observed with 100% to combination of CRX- CFZ-MEM and least MDR of 25% to CRX-CTR-CTX-CFZ-MEM-TET-CHL (Table 2).

Table 1. Antibiogram of *Edwardsiella tarda*

Antimicrobial class	Antimicrobial agent	Disk Potency	Sensitivity (%)		
			Sensitive (%)	Intermediate (%)	Resistance (%)
<b>Tetracyclines</b>	Tetracycline	30 µg	8.3	16.7	83.3
<b>Glycopeptides</b>	Vancomycin	30 µg	<i>Not available for Entrobacteriaceae</i>		
<b>Carbapenem</b>	Meropenem	10 µg	0.0	0.0	100.0
<b>Cephalosporins (2<sup>nd</sup> Generation)</b>	Cefuroxime	30 µg	0.0	0.0	100.0
<b>Cephalosporins (3<sup>rd</sup> Generation)</b>	Ceftriaxone	30 µg	8.3	58.3	33.3
	Cefotaxime	30 µg	0.0	8.3	91.7
	Ceftazidime	30 µg	0.0	0.0	100.0
<b>Potentiated Sulphonamide</b>	Trimethoprim-sulfamethoxazole (Cotrimoxazole)	1.25/23.75µg	41.7	8.3	50.0
<b>Aminoglycoside</b>	Amikacin	30 µg	75.0	8.3	16.7
	Gentamycin	10 µg	66.7	0.0	33.3
<b>Floroquinolone</b>	Ciprofloxacin	5 µg	91.7	0.0	8.3
<b>Phenicol</b>	Chloramphenicol	30 µg	58.3	16.0	25.0

**Table 2.** Multi-Drug Resistance Pattern for *Edwardsiella tarda*

Resistance Antibiotics	Number of resistance isolates (%)
CRX- CFZ-MEM	100.0
CRX-CTR-CTX-CFZ-MEM	91.7
CRX-CTR-CTX-CFZ-MEM-TET	83.3
CRX-CTR-CTX-CFZ-MEM-TET-COT	50.0
CRX-CTR-CTX-CFZ-MEM-TET-COT-GEN	33.3
CRX-CTR-CTX-CFZ-MEM-TET-CHL	25.0

**Figure 1.** XLD plate showing presumptive *E.tarda* colonies on showing varied size. They appeared small transparent with black centers to predominantly black colonies

#### 4. Discussion

Isolation of *Edwardsiella tarda* in an environment could be due to factors such as: high temperature, poor water quality and high organic content (10,17). *Edwardsiella tarda* is considered one of the most important bacteria that causes severe economic losses due to morbidity and mortality among various populations and age groups of fish in many countries (10,28). A prevalence of 62.5% observed for *Edwardsiella tarda* in this has revealed the presence of *E. tarda* O.

*niloticus* in Ibadan as well as established that it is culturable from apparently healthy fish, hence *O. niloticus* could serve as a possible source of infection to humans; as previously established by Castro *et al.*, 2014 (29). The observed prevalence from this study contrast the reports from the study of Eman *et al.*, 2018 (10) and Korní *et al.*, (2012) (30) who recorded the prevalence of *E. tarda* from *O. niloticus* to be 13.33% in Egypt, and reports from India (13.2 %), China (29.4%) and Brazil (6.6%) (9,31). Aside the environmental factors that favour the prevalence of *E. tarda*, diseased fish with or without any clinical signs of disease are equally important as many of them asymptotically carry pathogens, bearing risks of spreading diseases to other species including humans (8,10,14). Therefore, detection of these pathogens is crucial for their effective prevention and control of *E. tarda* and infections caused by them (29).

The resistance of *E. tarda* isolates obtained from *O. niloticus* in this study were observed to be 100% to: Ceftazidime, Meropenem, Cefuroxime; high resistance to tetracyclin, and cotrimoxazole and sensitivity to Ciprofloxacin, Chloramphenicol, Gentamicin and Cefotaxime. The observations of these drugs suggest their usage in aquaculture, and further reiterate the human risks associated AMR from them. These observations are similar to the reports of Eman *et al.*, 2018 (10). The Multi-Drug Resistance (MDR) pattern observed in this study, especially to the commonly used antibiotics in fish farms such as Cotrimoxazole and Tetracycline is similar to the reports of Eman *et al.*, 2018 (10). The MDR pattern observed evidences *E. tarda* as a multi resistant bacterium, representing a potential threat as a zoonotic pathogen of public health importance; these findings are similar to the reports of Eman *et al.*, 2018 (10). However, this study observed sensitivity of *E. tarda* to Ciprofloxacin, Chloramphenicol, Gentamicin and Cefotaxime; evidencing that these drugs are effective for controlling infections caused by this pathogen. This observation is in agreement to reports by Sahoo and Mukherjee, (1997)(32); and Eman *et al.*, (2018) (10). Multi-drug resistant (MDR) is a global public health dilemma that have been stated to have culminated in outbreaks of major global epidemics (Crump *et al.*, 2015). High resistance to antimicrobial agents could be translation of the misuse or abuse of the agents in the environment (33). This report therefore confirms microbial resistance

evidencing transfer risks of resistant bacteria to human through consumption of aquaculture products (34).

Antibiotic resistance menace and associated resultant health implications have been on the increase globally, and while most developing countries are the worse affected; because there are several situations, and human attitudes that support the development and spread of resistant microbes; such as inappropriate drug administration, especially, among farmers (35). Due to the economic situations of many developing nations, with resultant unaffordable diagnostic techniques and consultation fees, the prescription of broad spectrum antibiotics being against a wide range of different microbes have become a strategy to the control and prevention of major human and animal infections. Most of these antibiotics have resulted in making human and animals more vulnerable to more bacterial infections. Consequently, resulting in the risk of dangerous side effects, super infections and the development selection of drug resistant mutants (36).

Two theories have been advanced to explain the emergence of resistance in wild waters, i.e. resistant bacteria contaminating water from human or livestock sources; or antibiotic residues escaping into the water exposing the resident bacteria to low levels of antimicrobial agents overtime (34). Transfer of antimicrobial resistant bacteria to wild water due to sewage contamination has been reported in Australia (34,37). The resisted antibiotics are those commonly used for treatment of various ailments in humans, thus strongly supporting the theories of introduction into the aquatic ecosystems from human associated sources.

## 5. Conclusion

This study aimed at detecting, and determining the prevalence, and antibiogram of *Edwardsiella tarda* from *O. niloticus* sold in Ibadan, Oyo State. The study shows the Isolation of *E. tarda* from Tilapia sold in Ibadan, indicating that Tilapia harbour and serve as a source of infection for fish consumers and handlers. Furthermore, this study has also elucidated susceptibility and resistance characteristics of isolated *E. tarda* and human risks associated with consumption of fish. Based on the aforementioned conclusions, further studies are required to understand and characterize *E. tarda* strain found in this study. This would be useful for the determination of the virulent

factors of the bacteria and understanding its pathogenesis. The resultant effects of these would germane for designing preventive and control measures such as vaccine development against the occurrences of diseases associated with *E. tarda* both in humans and aquaculture. Furthermore, this study confirmed the occurrence as well as its resistance patterns of *E. tarda* in the study areas and since these bacteria are of public health importance, awareness should be created among fish farmers and stakeholders on possible health hazards accompanying handling and/or consumption of fresh and undercooked fish as well as drug usage. A comprehensive epidemiological study of *E. tarda* in Nigeria is highly recommended and systems for monitoring contamination levels of other zoonotic pathogens should be instituted. Further researches and studies of aquatic animal health and zoonosis should be adapted and employed as part of the tools for controlling diseases of public health importance. Regulatory policies for antibiotic usage, and standard operating practices for hygienic practices in the fishing and aquatic environments, controlled human activities, fishing procedures and handling of fish should be instituted and be followed by fishers and farmers to avoid disease outbreaks.

## Conflicts of Interest

Authors have no conflict of interest.

## Acknowledgment

We wish to appreciate all the participants involved in this study, and the department of Department of Veterinary Public Health and Preventive Medicine University of Ibadan, Nigeria.

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