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Molecular Characterization and Antibiotic Susceptibility of *Edwardsiella tarda* isolated from Farmed Nile Tilapia and African Catfish from Wakiso, Uganda

M. Nantongo[1, 3, *], **E. M. Mkupasi**[1], **D. K. Byarugaba**[2], **S. P. Wamala**[2], **R. H. Mdegela**[1], **J. K. Walakira**[3]

[1] College of Veterinary and Medical Sciences, Sokoine University of Agriculture, Tanzania.
[2] College of Veterinary Medicine, Animal Science and Biosecurity, Makerere University.
[3] National Fisheries Resources Research Institute-NARO, Uganda.

*Corresponding author. myayeri15@gmail.com

Abstract. This study was conducted to isolate and characterize *Edwardsiella tarda* (*E. tarda*) and assess its antimicrobial susceptibility. The bacterium was isolated in Wakiso District, Uganda, from symptomatic and asymptomatic Nile tilapia and African catfish raised in earthen ponds, tanks and cages between September 2016 and February 2017. The bacterium was then identified using conventional biochemical tests and API 20E test kits and characterized by sequencing 16S *rRNA* gene. The antibiotic susceptibility of 16 drugs was established using the Kirby BeurDisc diffusion method. Eight *E. tarda* isolates were identified using conventional biochemical tests but only one isolate was confirmed to be *E. tarda* by PCR. Phylogenetic analysis indicated a distant relationship with other 16S *rRNA* gene sequences retrieved from the GenBank. Six virulence genes (*CitC*, *muk*, *gadB*, *katB*, *esaV*, and *fimA*) that enhance bacterial survival and pathogenesis in the host were detected. The isolate registered low levels of antibiotic resistance as it was resistant only to Oxacillin, Vancomycin and Penicillin, to which it is intrinsically resistant. This implies low antibiotic usage in aquaculture in the area. Despite its low occurrence, presence of virulent genes in *E. tarda* indicates its potential to affect fish and human health.

Keywords: Aquaculture, *Edwardsiella tarda*, Phylogeny, Virulence

Introduction

Aquaculture in Uganda is increasing with a production of about 101659 tonnes (FAO, 2018). However, intensification of fish farming to meet the increasing fish demand is challenged with disease outbreaks (Bondad-Reantaso *et al.*, 2005). There is insufficient information on the type of fish pathogens affecting aquaculture systems in Uganda thus making management of disease outbreaks difficult. Fish diseases are known to cause economic losses, reduced growth and increased cost of production (Faruk *et al.*, 2004).Bacteria affect the health of both wild and

farmed fish and *Edwardsiella* species are known pathogens affecting fish populations worldwide (Austin and Austin, 1987; Abowei & Briyai, 2011; Park *et al*., 2012).

Edwardsiella tarda is the causative agent of Edwardsiellosis in fish, characterized by systemic hemorrhagic septicemia, internal abscesses and skin lesions (Ewing *et al*., 1965). The bacterium is opportunistic in nature, and disease outbreaks occur when poor environmental conditions prevail like overcrowding, poor water quality conditions, high organic content and high temperature (Mohanty and Sahoo, 2007). These factors influence the production of substances such as haemolysin, siderophore, superoxide dismutase, extracellular products and intracellular components that contribute to *E. tarda* pathogenicity and also expression of the two protein secretion systems that are involved in *E. tarda* virulence (Hossain *et al*., 2010; Guijarro*et al*., 2015; Huicab-Pech *et al*., 2016).

Occurrence of *Edwardsiella* species in farmed Nile tilapia and African catfish has been reported in Uganda but partially characterized to genus level (Walakira *et al*., 2014). Its prevalence in aquaculture systems is reported to vary with ponds not exceeding 5%, and tanks ranging between 50-70% (Mohanty and Sahoo, 2007; Joh *et al*., 2010). *Edwardsiella tarda* is also zoonotic causing gastroenteritis and skin lesions in humans (Park *et al*., 2012). *Edwardsiella tarda* is a facultative intracellular pathogen but its pathogenicity in host cells is not clearly understood (Xu and Zhang, 2014).However, its virulence factors are reported to enhance the bacterial survival, and pathogenesis in hosts. Therefore, identification of virulence-related genes is essential for explaining the pathogenesis of this bacterium.

Methods and Materials

The study was conducted in Wakiso District, central Uganda (0˚4´ S, 32˚45´ E) (Figure 1).

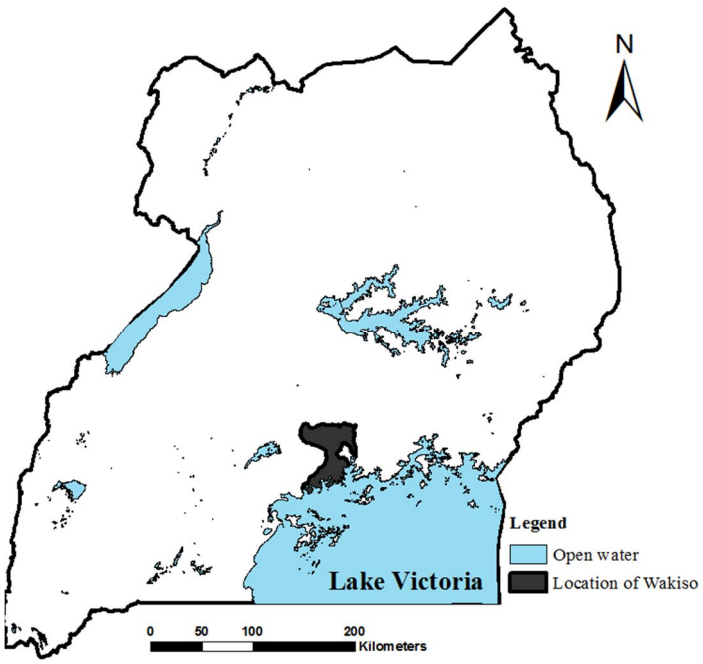

Figure 1. Map of Uganda showing the location of Wakiso District

Fish farming is one of the main economic activities in the district. Samples were collected from 17 active commercial and subsistence fish farms with ponds, tanks and cages. The study applied a cross-sectional study design. Fish farms were purposively selected, the inclusion criteria being history of disease outbreaks during the study period and willingness of the farmer to participate in the study. Simple random sampling technique was applied to select clinically healthy fish, whereas in case of disease occurrence, sick fish were purposively selected from the culture system.

Healthy and sick fish samples were collected and transported in sterile containers to the laboratory between September 2016 and February 2017. Necropsy on fish samples was conducted to examine external and internal lesions following procedures described by Noga (2010).

Samples of organs (liver, spleen and kidney) and swabs from gills and skin were collected and subjected to bacteriological examination following methods described by Noga (2010) Samples were homogenized, inoculated on Xylose Lysine Desoxycholate (XLD) agar plates and incubated at 28˚C for 36 hours after which they were examined for primary cultures. A well-differentiated single black colony was picked and sub cultured on XLD agar to produce a pure culture which were subjected to various biochemical tests.

Identification of the bacterium was done using cultural and morphological characteristics, conventional biochemical tests and Analytical Profile Index (API 20E) system (BioMerieux, France) according to manufacturer's instructions and the biochemical profiles of the isolates from API 20E were determined. The isolates were preserved in cryovials containing Brain Heart Infusion broth and glycerol and then stored in the freezer at -4˚C until further testing.

Genomic DNA extraction was done using QIAamp DNA mini kit (Qiagen) following manufacturer's instructions. DNA concentration was measured using NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc). The extracted DNA was preserved and stored at -20˚C.

The 16S *rRNA* gene was amplified by Polymerase Chain Reaction (PCR) using the 16S universal bacteria primers 27F (5' -AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') with the expected amplicon size of 1465bp (Lane 1991) from Invitrogen, Thermo Fisher Scientific (Waltham, MA USA). Polymerase Chain Reaction was performed in a thermo cycler (iCycler from Bio-Rad, United States of America). Each PCR reaction was performed in a final volume of 25µl containing 2.5µl of 10× reaction buffer (50 mM KCl, 75 mM Tris-HCl (pH 9.0), 2mM $MgCl_2$, 20mM $(NH_4)_2SO_4$), 0.5µl 10 mM deoxyribonucleotide mix, 0.2µl of *Taq* DNA polymerase, 1µl 10 mM of each forward and reverse primer, 2µl of DNA template and 16.8µl of sterile ultrapure water. PCR conditions included initial denaturation at 94˚C for three minutes, followed by 30 cycles of amplification as follows; denaturation at 94˚C for 30 seconds, annealing at 56˚C for 30 seconds and extension at 72˚C for two minutes. This was followed by a final extension step at 72˚C for five minutes and left to stand at 4˚C until collected for further analysis.

The PCR products were analysed on 1% agarose gel (Ultrapure agarose from Invitrogen, Thermos Fisher Scientific) at 100Volts for 60 minutes. The amplified DNA on the gel was visualized using Safe Imager™ from Invitrogen and bands of interest excised using a scalpel blade for gel purification. ChemiDoc™ XRS Molecular image (Bio Rad) was used for viewing and capturing gel pictures. The purified PCR products were sequenced with 16S 27F and 16S 1492R universal bacteria primers by Sanger sequencing techniques at GATC Biotech, Germany using ABI genetic analyser.

Both the forward and reverse sequences of the 16S *rRNA* gene were edited in Bioedit to generate a consensus sequence. Basic Local Alignment Search Tool (BLAST) searches were carried out in GenBanks particularly the National Centre for Biotech Information (NCBI). Related sequences were obtained and multiple sequence alignments were performed using ClustalW algorithm. The alignments were used to construct a phylogenetic tree using MEGA 7.0 (Kumar *et al*., 2016) using the Neighbour-Joining method (Saitou and Nei, 1987). The gene sequences used in phylogenetic analysis are shown in Table 1.

Table 1. Isolates whose sequences were used in phylogenetic analysis

SN	Name of culture	Accession number
1	*E. tarda* strain T1	KX388234.1
2	*E. tarda* strain C6	FJ607400.1
3	*E. tarda* strain UMT-WD-ON	FJ600537.1
4	*E. tarda* strain VMCU06	KU860461.1
5	*E. tarda* strain C7-5m	HQ663902.1
6	*E. tarda* strain ATCC 15947	JX866952.1
7	*E. tarda* strain 29-907R	KX828266.1
8	P. shigelloides ATCC 14029	M59159.1
9	*E. tarda* strain SY-ED14	KX388234
10	P. shigelloides ATCC 14029T	X74688.1
11	*E. tarda* strain 59-907R	KX828321.1
12	Salmonella enterica subsp enterica ATCC 13076	LSHA01000019.1
13	*E. coli* ATCC 11775	NZ_JMST01000035.1

Key: E = Edwardsiella, P = Pleisomonas

To characterize the virulence attribute of *E. tarda* isolate, seven virulence genes were screened for in this study. These included; *gadB, muk, citC, fimA, esrB, katB* and *esaV*. The genes were amplified by PCR using primers in Table 2.

Table 2. Primers used in the amplification of the virulence genes

Target gene	Sequence 5'⟶3'	Product size (bp)	Source
gadB (F)	5′- ATTTGGATTCCCGCTTTGGT-3′	583	Wang *et al.* (2012)
gadB (R)	5′- GCACGACGCCGATGGTGTTC-3′		
muk (F)	5′- TTGCTGGCTATCGCTACCCT-3′	357	Wang *et al*. (2012)
muk (R)	5′- TTGCTGGCTATCGCTACCCT-3′		
citC (F)	5′- TTTCCGTTTGTGAATCAGGTC-3′	591	Wang *et al*. (2012)
citC (R)	5′- AATGTTTCGGCATAGCGTTG-3′		
fimA (F)	5′- CTGTGAGTGGTCAGGCAAGC-3′	441	Wang *et al*. (2012)
fimA (R)	5′- TAACCGTGTTGGCGTAAGAGC-3′		
esrB (F)	5′-TCGTTGAAGATCATGCCTTGC-3′	311	Wang *et al.* (2012)
esrB (R)	5′-TGCTGCGGGCTTTGCTT-3′		
katB(F)	5′-CTTAGCCATCAGCCCTTCC-3’	1417	Wang *et al.* (2012)
katB(R)	5′-GCGAGTGCCGTAGTCCTT-3’		
esaV (F)	5’-GGTCAATAGCTGGCTACACAA-3’	955	Li *et al.* (2010)
esaV (R)	5’-GCGCCTCAGCGAGTATGCGAT-3’		

PCR reaction was performed in a final volume of 25µl containing 0.25µl of 10× reaction buffer (5 mM KCl, 7.5 mM Tris-HCl (pH 9.0), 0.2mM $MgCl_2$, 2mM $(NH_4)_2SO_4$), 0.05µl 1 mM deoxyribonucleotide mix, 0.02µl of Taq DNA polymerase, 0.1µl 1 mM of each forward and reverse primer, 0.2µl of DNA template and 1.68µlof sterile ultrapure water.

The PCR program was as follows; initial denaturation at 94°C for three minutes, followed by 32 cycles of denaturation at 94°C for one minute, annealing at 55°C for *citC, muk, esrB*, 58°C for *katB*, 57°C for *gadB*, and 60°C *fimA* for one minute, extension 72°C for one minute; and final extension at 72°C for ten minutes and left to stand at 4°C until further analysis.

PCR products were run on 1% agarose gel at 100Volts for 60 minutes. The amplified products on the gel were observed and captured using ChemiDoc™ XRS Molecular image (Bio Rad).

Drug susceptibility of the isolate to various commonly used antibiotics was done using the Kirby Beurdisc diffusion method according to Hudzicki (2009).Standard antibiotic discs (Hardy diagnostics, USA) containing Chloramphenicol (30 µg), Cefazolin (30 µg), Gentamycin (10 µg), Ceftriaxone (30 µg), Oxacillin (1 µg), Nalidixic acid (30 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Erythromycin (15 µg), Vancomycin (30 µg), Cefotaxine (30 µg), Ampicillin (10 µg), Trimethoprim-sulfamethoxazole (25 µg), Amoxillin/Clav. Acid (30 µg) and Penicillin (10 µg) were used. The isolate was cultured on nutrient agar and incubated at 25 °C for 24 hours after which single colonies were emulsified in physiological saline 0.85% to obtain a bacterial suspension, with turbidity that corresponded to 0.5 McFarland's standards (BioMerieux) that gives an approximate bacterial concentration of 1×10^6 CFU/ml. The standardized innoculum was evenly streaked on Mueller-Hinton Agar plates onto which different antibiotic susceptibility discs were placed and incubated at 25 °C for 24 hours. After, diameters of zones of inhibition were measured using a ruler in millimetres to determine the susceptibility of the isolate to the drugs, which was recorded as resistant, sensitive or intermediately sensitive according to CLSI/NCCLS standards (CLSI, 2006; CLSI, 2015).

Results

Clinical and Post Mortem Examination

A total of 81 Nile tilapia and 30 African catfish including healthy, moribund and recently died fish were collected from selected fish farms in Wakiso district, Uganda between September 2016 and February 2017. Of the examined fish, one Nile tilapia had ascites and petechial abdominal haemorrhages whereas three African catfish showed signs of abnormalities including petechial haemorrhages on the skin and fins, lordosis, a pale liver and ulcers on the belly and opercula region (Figure 2).

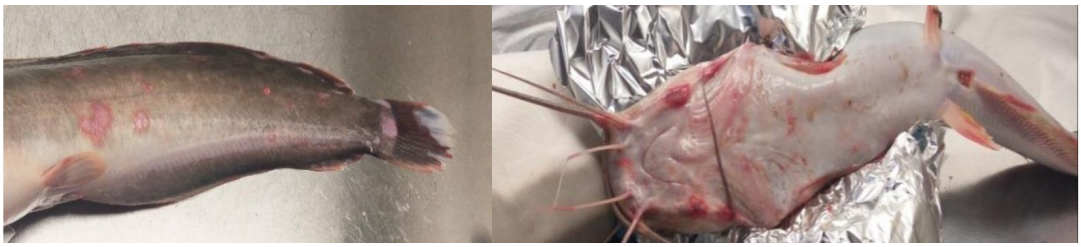

Figure 2. Haemorrhages and ulcers on farmed African catfish (*Clarias gariepinus*)

Phenotypic and Genotypic Identification

Out of 111 fish samples, eight *E. tarda* suspect isolates were identified from four Nile tilapia and four African catfish using conventional biochemical tests. On culturing, clear colonies with black centres surrounded with reddened media were produced on Xylose Lysine Deoxycholate (XLD) media after 36 hours at 28° C expected to be *E. tarda*.

Microscopic examination of the gram-stained smears of the colonies revealed gram negative bacilli and the wet mounts of these colonies showed motile rods. Results of the biochemical tests are shown in Table 3. The results showed that the isolates were biochemically homogenous except for variations in the MR and VP tests as only two isolates tested positive for MR and negative for VP.

Table 3. Phenotypic properties of the eight suspect *Edwardsiella tarda* isolates

Test	Isolate IDs							
	O.n 1	O.n 2	O.n 3	O.n 4	C.g 1	C.g 2	C.g 3	C.g4
Gram stain	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-
Indole production	+	+	+	+	+	+	+	+
Citrate	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+
Methyl Red	+	-	-	-	+	-	-	-
Voges-Proskaeur	-	+	+	+	-	+	+	+
Esculin hydrolysis	-	-	-	-	-	-	-	-
Gelatin hydrolysis	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-
Lactose utilization	-	-	-	-	-	-	-	-
H_2S production	+	+	+	+	+	+	+	+

Key: O.n - *Oreochromis niloticus*, C.g – *Clarias gariepinus*, + - positive result, -- negative result)

Only one isolate was identified to be *E. tarda* at 99.4% using the Analytical Profile Index (API) 20E kit with code number 4744000.The isolate tested positive for citrate utilization unlike in the conventional biochemical tests. The seven isolates identified as *E. tarda* in the biochemical tests were identified by the API 20E kit as *Plesiomonas shigelloides*, which is a fish pathogen belonging to family Enterobacteriaceae (Austin and Austin, 2007).

Identification of the suspected *E. tarda* isolate was further validated by amplification of the 16S *rRNA* gene sequencing and BLAST searches.

Phylogenetic Analysis

Comparison of the *E. tarda* isolate with known 16S *rRNA* sequences in the GenBank database using the BLAST program showed that the isolate had a 99.9% similarity to those of other members of *E. tarda* (Figure 3).

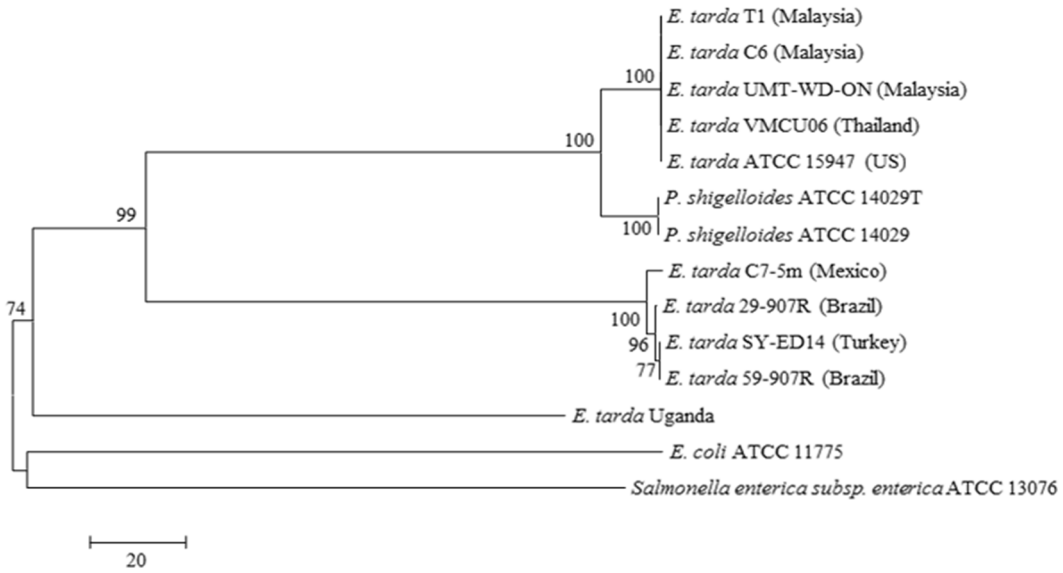

Figure 3. Molecular phylogenetic tree for the genetic relationship among the isolates based on the nucleotide sequence of the 16S *rRNA* gene

Note: The bootstrap process was repeated 500 times and the values are shown at major nodes in the tree. The scale bar is for a genetic distance marker (number of replacement nucleotides per site).

The taxonomic status, strain collection numbers and GenBank accession numbers of strains used in phylogenetic analysis are shown in Table 1.The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985).

Virulence genes harboured by Edwardsiella tarda

Six genes namely; *CitC, muk, gadD, esrB, katB* and *fimA* of the seven virulence genes screened for were present in the *E. tarda* that was isolated in this study (Figure 4).

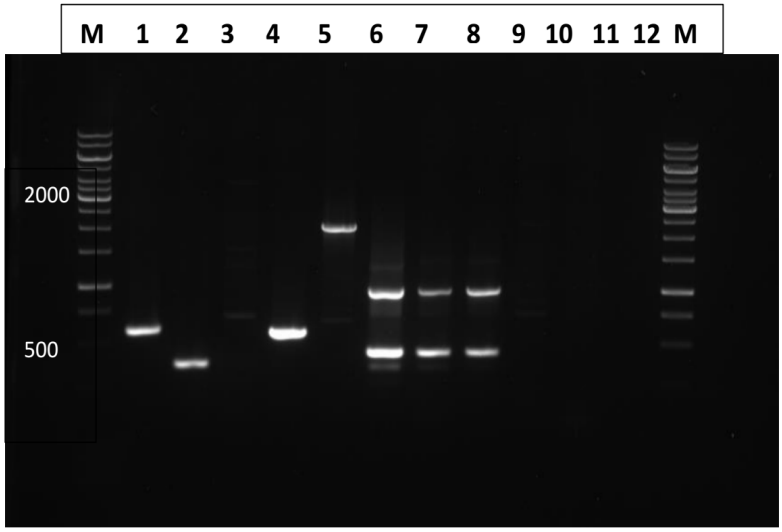

Figure 4. Agarose Gel electrophoresis of amplified virulence genes for the isolated *E. tarda*

Key: Lanes M - DNA marker, lane 1 -*citC*, lane 2 -*muk*, lane 3 -*esrB,* lane 4 -*gadB,* lane 5 -*katB,* lanes 6, 7 and 8 -*fimA* and *esaV*, Lanes 9 to 12 - blank

Antibiotic susceptibility of the isolate

It was found that the isolate was resistant to Oxacillin, Vancomycin and Penicillin, intermediately sensitive to Tetracycline and Erythromycin whereas it was sensitive to Chloramphenicol, Cefazolin, Gentamycin, Ceftriaxone, Nalidixic acid, Ciprofloxacin, Imipenem, Cefotaxime, Ampicillin, Trimethoprim-sulfamethoxazole and Amoxycillin (Table 4).

Table 4. Antibiotic susceptibility of the *E. tarda* isolate

Antibiotic discs	Disc code	Concentration (µg)	Status
Chloramphenicol	C	30	S
Cefazolin	CZ	30	S
Gentamycin	GM	10	S
Ceftriaxone	CRO	30	S
Oxacillin	OX	1	R
Nalidixic acid	NA	30	S
Tetracycline	Te	30	I
Ciprofloxacin	CIP	5	S
Imipenem	IPM	10	S
Erythromycin	E	15	I
Vancomycin	Va	30	R
Cefotaxime	CTX	30	S
Ampicillin	Am	10	S
Trimethoprim-sulfamethoxazole	SXT	25	S
Amoxillin/Clav. Acid	AmC	30	S
Penicillin	P	10	R

Key: S - sensitive, R - resistant, I - intermediate

Discussion

This is the first report on *Edwardsiella tarda* as a fish pathogen in Uganda. The information generated on virulence genes and its susceptibility to selected antibiotics is important for its management in aquaculture systems.

Although there is limited information about the disease occurrence in Uganda, *Edwardsiella tarda* is a significant pathogenic bacterium known to cause Edwardsiellosis in fresh and marine fish species both cultured and wild fish (Bullock and Herman, 1985; Mohanty and Sahoo, 2007). The disease leads to economic losses due to massive mortalities and reduced marketability of infected fish across the world (Faruk, 2004; Abraham *et al*., 2015). The present study established the occurrence of *E. tarda* infection at a rate of 7.2% by bacteriology and molecular technique confirmed *E. tarda* at a rate of 0.9% in Nile tilapia and African catfish farmed in Wakiso District in Uganda. A prevalence of 8.3% at farm level by bacteriology and conventional biochemical tests has been reported, suggesting involvement of the bacterium in disease occurrence in the country (Wamala *et al.,* 2018). Similar findings were reported in Ethiopia where occurrence of *E. tarda*-like species was 7.6% from clinically healthy tilapia (Nemo *et al.,* 2017). The two studies suggest difficulties in differentiating the bacterium from other phenotypically and biochemically related bacteria. In this study, molecular identification of the isolates suggests that bacteriological and convention biochemical methods may be less specific in identifying *E. tarda* and related bacteria to species level. In that regard, *E. tarda* positive cases reported basing on these phenotypic methods might be *E. tarda* like species.

Pathological changes observed in the *O. niloticus* that had the confirmed *E. tarda* isolate were ascites and petechial haemorrhages. The observed pathological lesions were similar to what is reported for *E. tarda* infections in fish elsewhere (El-Refaey, 2013; El-Seedy *et al*., 2015). However, edwardsiellosis clinical signs and pathological lesions are not reliable at arriving to a conclusive diagnosis as several bacterial infections present with similar features (Mohanty and Sahoo, 2007). Furthermore, infected fish with or without any clinical signs of disease are equally important as they may carry pathogens resulting into a risk of spreading the disease to other species including humans. Therefore, detection of these pathogens is crucial for their effective prevention and control (Castro *et al*., 2014).

In the current study, basing on the culture and morphological examination, the findings were suggestive of *E. tarda* species similar to observations made by other researchers who isolated *E. tarda* from cultured freshwater tilapia, African catfish, chinook salmon and sharp snout sea bream (El-Refaey, 2013; Katharios *et al*., 2015; Abraham *et al*., 2015; Eman *et al*., 2016) indicating no variation in the phenotypic characters of the pathogen.

Regarding the biochemical findings, generally were similar to observations made by other studies for *E. tarda* organisms (Joh *et al*., 2010; El-Refaey, 2013; El-Seedy *et al*., 2015; Griffin *et al*., 2013; Abraham *et al*., 2015; Eman *et al*., 2016). However, there were variations in the MR and VP tests as only two isolates tested positive for MR and negative for VP as indicated in Table 3. These results were not in agreement with any literature and this could possibly suggest involvement of other species. However, it has been reported that variations in phenotypic characteristics do exist and being attributed to the presence or absence of plasmids that control the metabolic traits of the phenotypic characteristics of the isolates (Acharya *et al*., 2007; Das *et al*., 2014).

One of the key finding from this study was the identification of *P. shigelloides* in most samples, in which both cultural and biochemical tests indicated *E. tarda* infection. This suggests that morphological and biochemical identification using conventional bacteriology techniques were

inconclusive in discriminating between *E. tarda* and *P. shigelloides.* API 20E kit test indicated only one isolate to be *E. tarda* at 99.4% of the eight isolates identified as *E. tarda* positive by the conventional biochemical tests. According to Buller (2004), differences in reactions occur between the API system and conventional biochemical tests and this is especially reported in decarboxylases, citrate, urea, indole and Voges-Proskeur tests. However, API test kits also have their limitations in bacteria identification that more definitive identification techniques such as PCR and sequencing are quite often preferred.

Phylogenetic analysis done in the current study separated the *E. tarda* strains in two groups without placing *E. tarda* isolated in this study in any group (Figure 3). One group clustered with *P. shigelloides* with high bootstrap support (100%). *Pleisomonas shigelloides* is deep rooted in the family Enterobacteriaceae and is aligned closer to *E. tarda* (Janda *et al*., 2016).Hence, this could explain the possible misidentification of *E. tarda* on biochemical tests as could have been the case in the current study and probably some other studies elsewhere. The second group shares greater similarity to the *E. tarda* isolate from this study with bootstrap support of 74% than the first group. These findings concur with those of other comparative phylogenomic studies that identified two distinct genetic groups of *E. tarda* (Panangala *et al*., 2006; Griffin *et al*., 2013). This finding therefore, suggests that the *E. tarda* isolate from this study is genetically distant from those from the GenBank that were included in this study.

In our study, six of the known *E. tarda* virulence genes screened for were present in the isolated *E. tarda* which were; *citC, muk, gadB, katB, esaV* and *fimA*. Two of these genes (*muk* and *gadB*) were also detected by Eman *et al.* (2016) and are believed to be specific to pathogenic *E. tarda*. According to literature, detected genes in this study are regarded as main virulence genes. *gadB* and *katB* provide resistance to the bacterium against host phagocyte killing activity, *fimA* enables cell adhesion, intracellular survival and replication of the bacterium in the host and *muk* is a putative killing factor (Mohanty and Sahoo, 2007; Katharios *et al.,* 2015). It was also suggested that the presence of *fimA* gene indicates the ability of *E. tarda* to bind to specific receptors in fish hence defining the site of entry and colonization. The most important properties for initiation of *E. tarda* infection process is the presence of surface structures which mediate motility, adherence and pathogen-host recognition. (Wang *et al.,* 2009). This implies that the isolated *E. tarda* is potentially pathogenic to fish provided conducive environment prevails.

The detected virulence genes are known to be present only in virulent strains and therefore they are considered as biomarkers in the diagnosis of pathogenic *E. tarda*. These genes are used in determining how pathogenic bacteria interact with host to cause systemic infections and vaccine development as they are used in designing novel therapeutics and common antigens (Méndez *et al*., 2012). Although the current study did not assess the virulence of the isolate based on *in vivo* experiments, the presence of these virulence genes and observed clinical signs could indicate the presence of edwardsiellosis in farmed fish in Uganda.

Resistance to Vancomycin, Oxacillin and Penicillin was also observed by Wamala *et al.* (2018), however this is typical of all members of family Enterobacteriaceae (Stock and Wiedemann, 2001). This resistance is attributed to their outer membranes that resist permeability of these antibiotics. The anterior channel size of the porins is broader than the molecular size of Penicillin hence its hydrophobicity makes it impossible to cross the outer membrane (Stock and Wiedemann, 2001).

The isolate's sensitivity to Gentamycin, Nalidixic acid, Ciprofloxacin, Cefotaxime and Trimethoprim-sulfamethoxazole was also observed by Akinbowale *et al.* (2006).However, their study found *E. tarda* to be resistant to Ampicillin, Amoxycillin, Chloramphenicol, Tetracycline and Erythromycin. Abdel-Latif and Sedeek (2017) found, as in this study, *E. tarda* to be less

sensitive to Erythromycin and Oxytetracycline and sensitive to Gentamycin and Ciprofloxacin although, unlike in this study, they found the bacteria to be resistant to Ampicillin. Results of this study are in contrast to some studies for example Ogbonne *et al.* (2018) who found *E. tarda* to be resistant to Amoxicillin, Chloramphenicol and Nalidixic acid and intermediately sensitive to Gentamycin. This resistance could either be possibly attributed to the presence of plasmids or presence of naturally occurring resistant bacteria in the aquatic sediments. However, frequent use of antibiotics in Uganda has been reported in veterinary practices for treatment of infectious diseases, prophylaxis and feed additives (UNAS *et al.*, 2015) which could explain the resistance to Oxacillin, Vancomycin and Penicillin.

Overuse and misuse of antimicrobial agents lead to emergence of antimicrobial resistance in aquatic pathogens. Fortunately, results of this study show low acquired antibiotic resistance, which are in agreement with findings of Wamala *et al.* (2018).This could be because, according to UNAS *et al.* (2015), antibiotics are not widely used in Ugandan aquaculture for therapeutic purposes as their use is only for research and is strictly regulated.

Conclusion

This study confirmed occurrence of *E. tarda* in farmed Nile tilapia and African catfish in Uganda. Isolation and characterization of *E. tarda* from farmed fish is important information for the industry as effective prevention and control strategies for this pathogen can now be developed. Further studies should be conducted to understand the synergistic effect of *E. tarda,* and the zoonotic impact on consumers and fish practitioners.

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