Malaysian Journal of Microbiology, Vol 16(2) 2020, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.180304

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

High prevalence of carbapenem and extended spectrum β-lactam resistant *Escherichia coli* from Tilapia (*Oreochromis niloticus*) from two wet markets in Metro Manila, Philippines

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Received 13 November 2018; Received in revised form 5 August 2019; Accepted 13 September 2019

ABSTRACT

Aim: Multiple drug resistant bacteria are serious health problems worldwide, with carbapenem resistant and extended spectrum-β-lactamase (ESBL) producing Enterobacteriaceae classified by Centers for Disease Control and Prevention under the category of "Urgent Threats" and "Serious Threats", respectively. The study characterized *Escherichia coli* from *Oreochromis niloticus* procured from two wet markets in Metro Manila in January and September 2016 for their drug resistance.
Methodology and results: Antimicrobial susceptibility profiles were determined using standard disc diffusion method. Extended-spectrum β-lactamase production was confirmed using clavulanate double disc synergy assay, carbapenemase production was tested using modified Hodge test, and MBL (metallo-β-lactamase) production was tested using EDTA double disc synergy assay. Results show that of the 25 isolated *E. coli*, 24 or 96% were resistant to at least one antimicrobial, with 60% being multiple drug resistant. These strains exhibited 20 different resistance phenotypes, suggesting these were different strains. Fifteen of the isolates (60%) screened positive for ESBL. Among these, 11 lost their resistance, indicating the instability of the resistance genes in the host, a characteristic of plasmid-mediated ESBL production. The ESBL suspects tested were confirmed to be ESBL producers. A high 48% of isolates were found to be resistant to carbapenems, with eight of the 11 tested (73%) being positive for carbapenemase production. MBL positive isolates carried the bla_{IMP} gene as determined by multiplex PCR and nucleotide sequencing.
Conclusion, significance and impact of study: Study showed a high prevalence of multiple drug resistant *E. coli* isolates from the commonly-consumed Tilapia procured from the wet markets. This result is compounded by the alarmingly high prevalence of carbapenem resistant and ESBL-producing strains among these isolates. Considering that the genes coding for these resistances are found in mobile genetic elements such as plasmids and integrons that can be transferred to other bacteria resulting to a rapid increase in drug resistant strains, it is highly imperative for all the concerned government units to establish a well-coordinated national surveillance program to monitor and address the occurrence and increase in drug resistant microorganisms in man, animals and the environment. In addition, prudent use of antimicrobials among these should be seriously instituted.

Keywords: Multiple drug resistant Enterobacteriaceae, carbapenemase, *Escherichia coli*, extended spectrum β-lactamase, *Oreochromis niloticus*

INTRODUCTION

The rapid increase in the prevalence of multiple drug resistance (MDR) is a worldwide public health concern [Centers for Disease Control and Prevention (CDC), 2013]. Multiple drug resistance is defined as resistance of the organism to at least one or more agents in three or more classes of antimicrobial categories (Magiorakos *et al.* 2012). Included among the three categories of drug resistant bacteria established by the Centers for Disease Control and Prevention in 2013 are the carbapenem resistant Enterobacteriaceae (CRE) and the extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae, which are classified under the categories of "Urgent Threats" and "Serious Threats", respectively.

ESBL-producing bacteria are resistant to all penicillins, cephalosporins with the oxyimino side chain such as ceftazidime, ceftriaxone, cefotaxime, and the monobactam aztreonam, but are susceptible to

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(2) 2020, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.180304

carbapenems and cephamycin (Ghafourian *et al*., 2015). ESBL genes are found on plasmids and have been shown to be transferable to other bacteria through conjugation (Cabrera and Rodriguez, 2009). At the same time, these conjugative plasmids were shown in the same study to carry genes coding for resistances to other classes of antimicrobials such as to aminoglycosides, tetracycline, and sulfamethoxazole trimethoprim. On the other hand, MBLs hydrolyze almost all clinically available β-lactam antibiotics including the carbapenems, which is the last resort group of drugs for MDR Gram negative bacteria. The MBL genes are found in mobile integrons that usually harbor other resistance genes (Mojica *et al*., 2016). These integrons may be integrated into chromosomes or mobile plasmids. In the Philippines, data from the 2016 Annual Report of the Antimicrobial Resistance Surveillance Program of the Department of Health on Enterobacteriaceae isolates from hospital bacteriology laboratories in 24 sentinel sites located in 16 regions of the country show that 30% of *E. coli* were screened to be ESBL positive, while 4.1% were resistant to the carbapenem ertapenem, 4.2% were resistant to imipenem and 3.8% were resistant to meropenem (Department of Health Philippines, 2017). The resistance rate of 4.2% in 2016 for imipenem was found to be significantly higher when compared to 3.5% in 2015 (p= 0.0398).

Although prevalence of MDR Enterobacteriaceae isolates in the clinical setting is under close surveillance and is periodically monitored and reported, prevalence of MDR Enterobacteriaceae in seafood for human consumption has not been sufficiently highlighted despite their serious impact on public health (Yagoub 2009). Studies have fully demonstrated the isolation of pathogenic strains of *E. coli*, such as *E. coli* O157:H7, shiga-toxigenic *E. coli* (STEC), enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) from fish in wet markets (Ayulo *et al*., 1994; Kumar *et al*., 2001; Teophilo *et al.*, 2002; Hwang *et al*., 2004; Thampuran *et al.*, 2005; Ristori *et al*., 2007; Yagoub, 2009; Costa, 2013). Outbreaks involving these intestinal pathogens are of immense public health significance. The impact is unarguably worse if the strains involved are MDR strains.

Oreochromis niloticus, which is commonly called Tilapia in the vernacular, is one of the favored fishes in the Philippines, and is a favorite aquaculture commodity. However, antimicrobial-supplemented feeds are still rampantly used in fish farms. Various studies have shown the positive selective pressure imposed by this practice on the increase in drug resistant bacterial flora found in aquaculture food products (Ciceron *et al.*, 2008; Sapkota *et al*., 2008; Alexander *et al.*, 2010; Ryu *et al.*, 2012; Berglund, 2015). These drug resistant bacteria can contaminate the environment, infect individuals working in the aquaculture facilities, other handlers such as vendors or the consumers through the food chain (Sapkota *et al.* 2008). The study thus aimed to determine the antimicrobial susceptibility profiles of *E. coli* obtained from the gills and intestines of Tilapia procured from two wet markets in Pasay and Manila, and characterize them for carbapenem resistance and ESBL production. The findings from this study are expected to mobilize further studies on drug resistant foodborne bacteria associated with aquaculture produce. At the same time, the results can serve to rationalize the regulation of antimicrobial use for growth and prophylaxis in the aquaculture and livestock industry.

MATERIALS AND METHODS

Procurement of Tilapia for Isolation of *E. coli*

Thirty Tilapia samples were procured from Cartimar Market, Manila in January 2016, and another 30 Tilapia from San Andres Market, Manila in September 2016. These are two of the biggest wet markets located in Manila, and residents in this area and nearby places commonly purchase their seafood from these markets due to their easy accessibility and wide variety of food products. The Tilapia sampling was conducted in the morning during which time the produce for sale are freshly delivered. According to the vendors, the Tilapia were from Batangas province. The samples were separately wrapped, sealed, and transported in an ice chest to the laboratory, and individually processed for *E. coli* isolation. These were aseptically dissected, and the gills and the intestinal lumen were swabbed and inoculated into MacConkey agar and EMB (Eosin Methylene Blue) agar plates, which were incubated at 37 °C for 16 to 18 h for morphological characterization of their colonies. Lactose fermenting colonies typical of *E. coli*, i.e. growth of brick red or pink colonies on MacConkey agar and dark colonies with greenish metallic sheen on EMB agar, were purified and identified based on the reactions in TSI (Triple Sugar Iron), indole, MR (Methyl Red), VP (Voges Proskauer), Simmon's citrate agar and urease production test.

Antimicrobial susceptibility testing

The standard disc diffusion method (Clinical and Laboratory Standards Institute (CLSI), 2015] was used to determine the antimicrobial susceptibility of the isolates. Three to five colonies per plate from a 16 to 18 h plated culture were transferred into tubes with sterile distilled water. The turbidity was adjusted to equal that of 0.5 MacFarland standard to approximate 1.5 x10^8 CFU mL^{-1}.

The prepared inoculum was swabbed onto Mueller Hinton agar plates. Antibiotic discs with CIP (Ciprofloxacin) (5 µg), AMP (Ampicillin) (10 µg), C (Chloramphenicol) (30 µg), CAZ (Ceftazidime) (30 µg), CN (Gentamicin) (10 µg), TE (Tetracycline) (30 µg), MRP (Meropenem) (10 µg), CTX (Cefotaxime) (30 µg), ETP (Ertapenem) (10 µg), SXT (Sulfamethoxazole-trimethoprim) (25 µg), ATM (Aztreonam) (30 µg), IMI (Imipenem) (10 µg) and CRO (Ceftriaxone) (30 µg) were placed on the inoculated plates. After incubation for 18 h at 37 °C, the diameters of the zones of inhibition were measured and interpreted using the CLSI (2015) interpretative standard. Each test was done in triplicate.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(2) 2020, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.180304

Table 1: Sequences of primers for amplification of Metallo-β-lactamase genes in the study (Poirel *et al.,* 2010).

Primers*	Sequence (5' to 3')**	Gene	Product size (bp)
IMP F	GGAATAGAGTGGCTTAAYTCTC	*bla*$_{IMP}$	232
IMP R	GGTTTAAYAAAACAACCACC		
SPM F	AAAATCTGGGTACGCAAACG	*bla*$_{SPM}$	271
SPM R	ACATTATCCGCTGGAACAGG		
AIM F	CTGAAGGTGTACGGAAACAC	*bla*$_{AIM}$	322
AIM R	GTTCGGCCACCTCGAATTG		
VIM F	GATGGTGTTTGGTCGCATA	*bla*$_{VIM}$	390
VIM R	CGAATGCGCAGCACCAG		
GIM F	TCGACACACCTTGGTCTGAA	*bla*$_{GIM}$	477
GIM R	AACTTCCAACTTTGCCATGC		
BIC F	TATGCAGCTCCTTTAAGGGC	*bla*$_{BIC}$	537
BIC R	TCATTGGCGGTGCCGTACAC		
SIM F	TACAAGGGATTCGGCATCG	*bla*$_{SIM}$	570
SIM R	TAATGGCCTGTTCCCATGTG		
NDM F	GGTTTGGCGATCTGGTTTTC	*bla*$_{NDM}$	621
NDM R	CGGAATGGCTCATCACGATC		
DIM F	GCTTGTCTTCGCTTGCTAACG	*bla*$_{DIM}$	699
DIM R	CGTTCGGCTGGATTGATTTG		
KPC Fm	CGTCTAGTTCTGCTGTCTTG	*bla*$_{KPC}$	798
KPC Rm	CTTGTCATCCTTGTTAGGCG		232

* F is sense primer, R is antisense primer
**Y is C or T

Phenotypic confirmatory test for ESBL production

Escherichia coli isolates that screened positive for ESBL production, i.e., those that were found to be resistant to any of the following cephalosporin agents: aztreonam, cefotaxime, ceftazidime, and ceftriaxone were further tested for confirmation of ESBL production (CLSI, 2015). The procedure was as aforementioned except for the use of the cephalosporin antibiotic discs with and without 10 µg of clavulanate. An enhancement of the diameter of growth inhibition in the antibiotic disc with clavulanate, a β lactamase inhibitor, by > 5mm compared to the corresponding antibiotic disc without the clavulanate was indicative of ESBL production. Each test was done in triplicate.

Modified Hodge test for the determination of carbapenemase production

Carbapenem resistant *E. coli* isolates were tested to determine the production of carbapenemase following the method of CLSI (2015). *Escherichia coli* ATCC 25922 inoculum was prepared as described above for the disc diffusion assay, except that the turbidity-adjusted inoculum was further diluted 10^{-1}. It was swabbed on Mueller Hinton agar plate, and a carbapenem (10 µg) disc was placed at the center of the plate. Three to five colonies per plate from 16 to 18 hr culture of carbapenem resistant *E. coli* test isolates were streaked from the edge of the carbapenem disc to the periphery of the plate. A positive result is indicated by the appearance of cloverleaf like indentation after 18 to 24 h of incubation at 37 °C because the susceptible *E. coli* ATCC 25922 will grow over the areas where the carbapenemase secreted by carbapenemase-producing bacteria hydrolyzed the antibiotic. Each test was done in triplicate.

Metallo- β- lactamase production test

Carbapenem resistant *E. coli* isolates were tested for MBL production using the double disc synergy assay (Hammoudi *et al.*, 2014). The same procedure as the test for ESBL production was followed, except for the antibiotics and metal chelator used. These were comprised of imipenem (10 µg) with and without 10 µg of 0.5 M EDTA (pH 8.0). An increase in the diameter of growth inhibition in the imipenem disc with EDTA, by >_7 mm compared to the imipenem disc without EDTA was indicative of metallo-β-lactamase production. A control EDTA disc was tested along with the antimicrobial discs. Each test was done in triplicate.

Extraction of genomic DNA

DNA for the PCR reaction was extracted using the boiling method (Cabrera *et al.*, 2010). Studies have shown that DNA yield from boiling technique was up to 17 times higher than phenol-chloroform extraction technique (Oliveira *et al*., 2014). In addition to its lower cost, chemicals that present health risks such as phenol and chloroform are not used. Briefly, 3 to 5 colonies from 16-18 h culture of the bacterial isolate were thoroughly

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(2) 2020, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.180304

suspended in 100 µL of Tris-EDTA buffer (TE buffer, pH 8.0). This was then heated in a boiling water bath for 10 min and centrifuged at 13,000 rpm for 5 min. The supernatant was used as the source of DNA.

Multiplex PCR to detect MBL genes

Multiplex PCR was carried out following the method of article by Poirel (Poirel *et al.,* 2010) with modifications. The PCR mix was comprised of 1 µL of DNA template, 1.5 mM $MgCl_2$, 0.5 mM dNTP, 0.5 µM of each forward and reverse primers, 0.05 U/µL KAPA *Taq* DNA pol, 1X PCR buffer and ddH_2O. Three multiplex mixtures of different primer combinations were used. Number 1 mix comprised of primers for bla_{IMP}, bla_{VIM} and bla_{SPM}, Number 2 mix comprised of primers for bla_{NDM}, bla_{KPC}, bla_{BIC}, and Number 3 mix comprised of primers for bla_{AIM}, bla_{GIM}, bla_{SIM}, and bla_{DIM} (Poirel *et al.,* 2010). For the Number 3 mix, 3 µL dimethyl sulfoxide were added. Table 1 shows the sequences of the primers used and the expected amplicon sizes.

Amplification was done with thermocycler (MJ Research, Waltham MA, USA) with the following conditions: Initial denaturation at 94 °C for 5 mins, and 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min. Final extension was at 72 ˚C for 5 mins. The amplicons were sent to First Base Malaysia for sequencing.

RESULTS

Twenty-five *E. coli* were isolated from the gills (16 isolates) and intestines (9 isolates) of 22 Tilapias out of the 60 samples studied. *Escherichia coli* was isolated from both the gills and intestines of three Tilapia samples. Fifteen of the *E. coli* were from 13 fish samples procured from Cartimar Market, while the remaining 10 *E. coli* were from 9 fish procured from San Andres Market. Isolates with the following characteristics were identified as *E. coli*: pink colonies on MacConkey agar; dark colonies with greenish metallic sheen on EMB agar; acidic butt, acidic slant, positive for CO_2 and negative for H_2S in TSI; positive reactions in indole and MR tests, and negative reactions in VP, citrate and urease tests.

Antimicrobial susceptibility profiles of the *E. coli* isolates

The antimicrobial susceptibility profiles of the *E. coli* isolates were determined using the standard disc diffusion method. Table 2 shows that out of the 25 isolates, only one isolate was totally susceptible to all the test antimicrobials. Twenty-two isolates showed resistance to at least two antimicrobials, while the remaining two were resistant to ampicillin alone. Following Magiorakos *et al.* (2012) definition of multiple drug resistance, which is resistance to at least one antimicrobial in three or more classes of antimicrobial categories, results show that 15 of the 25 isolates (or 60%) were MDR strains. Table 2 also shows that there were 20 different resistance phenotypes among the 24 isolates resistant to at least one of the antimicrobials.

Figure 1 and Table 3 show the % isolates with resistance or intermediate responses to the different test antimicrobials. Twenty isolates (or 80%) showed resistant or intermediate responses to ampicillin, while 56% were resistant or intermediate to sulfamethoxazole trimethoprim. Particularly worth noting are the disturbing high 60% of the isolates that were resistant to any of the cephalosporins and/or aztreonam, i.e. ESBL suspects (Table 2), and the 48% isolates resistant (11 isolates) or intermediate (one isolate) to the carbapenem imipenem. In addition, six of the 11 imipenem resistant isolates were also resistant to the carbapenem ertapenem, and resistant or intermediate to meropenem, while two imipenem resistant isolates were also resistant to ertapenem (Table 2).

Production of ESBL

The 15 *E. coli* isolates that showed either resistant or intermediate responses to the β-lactam antibiotics with the oxyimino side chain, namely: ceftazidime, cefotaxime, and ceftriaxone, and to the monobactam aztreonam were tested further for the production of ESBL using the double disc synergy test with clavulanate. Eleven (11) of the 15 isolates that screened to be ESBL producers lost their resistances to these antimicrobials, signifying that the resistances were unstable in the isolates, and thus might be plasmid mediated. This is a characteristic of ESBL genes. The isolates tested were confirmed to be positive for ESBL production.

Production of carbapenemase and metallo β-lactamase (MBL)

Escherichia coli isolates that were resistant or had intermediate responses to any of the carbapenems, namely: imipenem, meropenem and ertapenem were tested for the production of carbapenemase using the modified Hodge test and the production of MBL using the double disc synergy assay. Table 4 shows the results, while Figure 2 shows representative assay plates. Of the 11 isolates tested (one isolate was not tested), six were positive for both carbapenemase production and MBL production, while two were positive for carbapenemase, but not for MBL production. One was negative for carbapenemase production but positive for MBL. Two of the carbapenem resistant isolates were negative in any of the tests. This suggests that the resistance was due to mechanisms other than the production of carbapenemase, such as the presence of efflux pumps and impermeability of the plasma membrane to the antimicrobials (Meletis *et al.*, 2012).

PCR Detection and Identification of MBL genes

Escherichia coli isolates that were shown to be positive

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(2) 2020, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.180304

Table 2: Antimicrobial resistance phenotypes of *E. coli* isolates from Tilapia obtained from two wet markets.

Isolate number/ Source*	Resistance Phenotype (including intermediate, I)	Number of Drug Categories to which Resistant or Intermediate	**MDR Magiorakos *et al.* (2012)
T. 45I/ SA	CIP, AMP, TE (I), SXT, CRO (I), CAZ, ETP, MRP (I), IMI	6	+
T. 57G/ SA	AMP, C (I), TE, SXT, CAZ, ETP, MRP, IMI	6	+
T. 54G/ SA	AMP, CRO (I), ATM, ETP, IMI, MRP (I)	4	+
T.34I/ SA	AMP, TE (I), CAZ, ETP, MRP, IMI	4	+
T. 43I/ SA	AMP, SXT, CRO (I), ATM, ETP, IMI	5	+
T. 42I/ SA	AMP, SXT, ETP, MRP, IMI	3	+
T. 25G/ C	AMP, SXT (I), ATM, IMI	4	+
T. 47I/ SA	AMP, SXT, ATM, IMI	4	+
T. 53G/ SA	AMP, SXT (I), CAZ (I), IMI	4	+
T. 16G/ C	AMP, CRO (I), CTX (I), CAZ	2	-
T. 42G/ SA	AMP, SXT (I), CAZ, ETP, IMI	4	+
T. 7G/ C	AMP (I), SXT, ATM	3	+
T. 17G/ C	AMP, SXT, ATM	3	+
T. 24I/ C	AMP, TE, SXT	3	+
T. 18G/ C	AMP, TE, SXT	3	+
T. 11G/ C	AMP, C, TE (I)	3	+
T. 23G/ C	AMP (I), CTX (I)	2	-
T. 22I/ C	AMP	1	-
T. 18I/ C	AMP (I)	1	-
T. 51G/ SA	SXT (I), ETP, MRP, IMI	2	-
T. 28I/ C	SXT, IMI (I)	2	-
T. 27G/ C	CRO (I), ATM	2	-
T. 26G/ C	CAZ, ATM	2	-
T. 12G/ C	ATM, TE (I)	2	-
T. 28G/ C	SUSCEPTIBLE TO ALL	0	-

Isolate number/ Source*: G is Gills; I is Intestine; SA is San Andres Market, Manila; C is Cartimar, Pasay City.
**MDR: non-susceptible to ≥1 agent in ≥ 3 antimicrobial categories.
AMP- ampicillin; CTX- cefotaxime; ATM- aztreonam; ETP- ertapenem; C- Chloramphenicol; IMI- imipenem; CAZ- ceftazidime; MRP- meropenem; CIP- ciprofloxacin; SXT- sulfamethoxazole trimethoprim; CRO- ceftriaxone; TE- tetracycline.

Table 3: Number and percentage of *E. coli* isolates with resistant or intermediate responses to the test antimicrobials (n=25).

Antibiotics	No. (%) resistant isolates	No. (%) intermediate isolates	No. (%) intermediate + resistant isolates
CIP	1 (4)	0 (0)	1 (4)
AMP	17 (68)	3 (12)	20 (80)
C	1(4)	1 (4)	2 (8)
CN	0 (0)	0 (0)	0 (0)
TE	3 (12)	4 (16)	7 (28)
SXT	10 (40)	4 (16)	14 (56)
CRO	0 (0)	5 (20)	5 (20)
CTX	0 (0)	2 (8)	2 (8)
CAZ	6 (24)	1 (4)	7 (28)
ATM	9 (36)	0 (0)	9 (36)
ETP	8 (32)	0 (0)	8 (32)
MRP	4 (16)	2 (8)	6 (24)
IMI	11 (44)	1 (4)	12 (48)

AMP- ampicillin; CN- gentamicin; MRP- meropenem; ATM- aztreonam; CRO- ceftriaxone; SXT- sulfamethoxazole trimethoprim; C- chloramphenicol; CTX- cefotaxime; TE- tetracycline; CAZ- ceftazidime; ETP- ertapenem; CIP- ciprofloxacin; IMI- imipenem

for MBL production using the double disc synergy assay were studied for the presence of the MBL genes. The bacterial isolates tested and the *bla*$_{IMP}$ reference strains showed the expected amplicon size of ~ 232bp for *bla*$_{IMP}$ (Figure 3). Analysis of the nucleotide sequence using NCBI BLAST confirmed the amplicons to be that of *bla*$_{IMP}$.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(2) 2020, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.180304

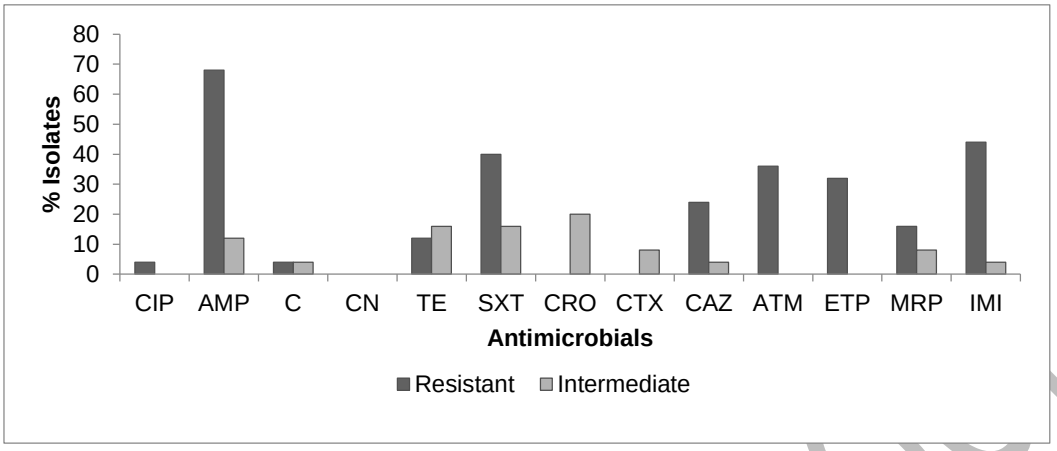

Figure 1: Percentage (%) *E. coli* isolates from Tilapia with resistant or intermediate responses to the test antimicrobials. AMP- ampicillin; CTX- cefotaxime; ATM- aztreonam; ETP – ertapenem; C- chloramphenicol; IMI- imipenem; CAZ- ceftazidime; MRP- meropenem; CIP- ciprofloxacin; SXT- sulfamethoxazole trimethoprim CN- gentamicin; TE- tetracycline; CRO- ceftriaxone.

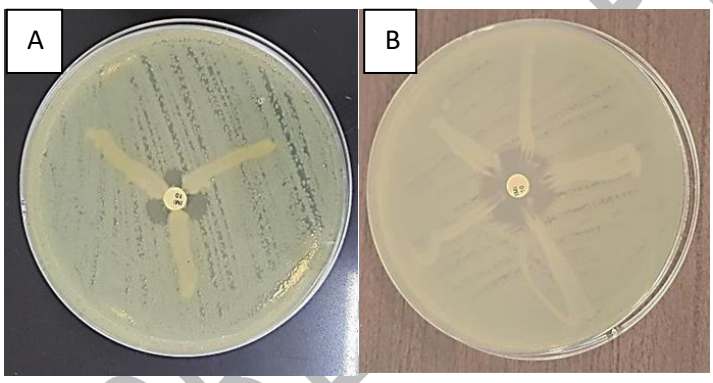

Figure 2: Results of Modified Hodge test for carbapenemase production. (A) Positive results shown by enhanced growth of ATCC 25922 around the streaks of three test isolates at the intersection of the streaks and the zone of inhibition (cloverleaf growth of *E. coli* ATCC 25922) (B) Negative results showing no enhancement of growth.

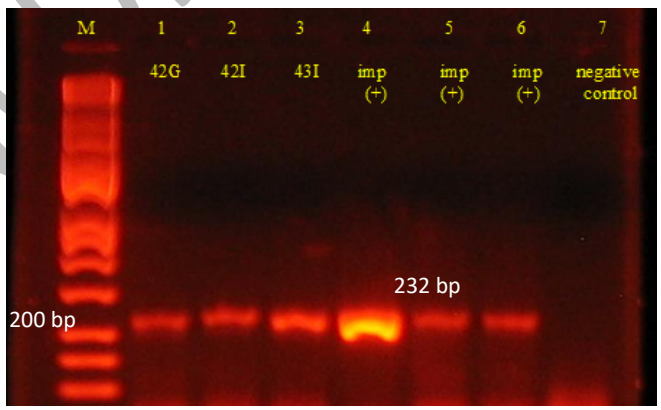

Figure 3: Multiplex PCR results after agarose gel (1.8%) electrophoresis at 50V. Lane M: DNA ladder; Lanes 1, 2, 3 are MBL positive *E. coli* isolates from Tilapia; Lanes 4, 5 and 6 are *bla*$_{IMP}$ positive controls. Lane 7 is the negative control. Expected amplicon size for *bla*$_{IMP}$ is 232 bp.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(2) 2020, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.180304

Table 4: Results of the assays for carbapenemase and MBL production for the carbapenem resistant isolates.

Isolate number	Carbapenemase	MBL
T 45 I	(+)	(+)
T 43 I	(+)	(+)
T 42 G	(+)	(+)
T 42I	(+)	(+)
T 54 G	(+)	(+)
T 57 G	(+)	(+)
T 51 G	(+)	(-)
T 53 G	(+)	(-)
T 47 I	(-)	(+)
T 25G	(-)	(-)
T34 I	(-)	(-)

DISCUSSION

The high prevalence of drug resistant *E. coli* from the gills and intestines of Tilapia from wet markets is of serious public health concern. The study findings showed that 96% isolates were resistant to at least one antimicrobial, and 60% isolates were MDR strains following the classification of Magiorakos *et al*. (2012). The results are not consistent with those of Kathleen *et al*. (2016), which showed low level of antibiotic resistance in the 94 bacterial isolates belonging to 17 genera that were isolated from sediment, water, and cultured organisms (fish and shrimp) in selected aquaculture farms, except to ampicillin and streptomycin, which have been used in aquaculture for several decades. On the other hand, the results of Gufe *et al*. (2019) also showed a high percent of MDR isolates, where 59 of the 60 or 98% of the bacterial isolates from fish sold in markets, which were comprised of *E. coli*, *Staphylococcus aureus, Aeromonas* spp., *Enterobacter aerogenes, Proteus mirabilis, Citrobacter* spp*.,* coagulase-negative staphylococci, *Pseudomonas* spp., and *Klebsiella* spp. were MDR strains.

Furthermore, the study showed that a high 60% (15 out of 25 isolates) tested resistant to the extended spectrum-β-lactam antibiotics. The results are consistent with those of Singh *et al*. (2017), which detected the ESBL-positive phenotype in 78.60% (169 out of 215) of the Enterobacteriaceae isolated from fresh seafood sold in retail markets. This is of serious concern as it is known that hydrolysis of the antibiotics by ESBLs confers resistance to the penicillin, first, second and third generation cephalosporins and aztreonam (except cephamycin and carbapenems) (Paterson and Bonomo 2005). In addition, ESBL genes are often carried in plasmids that also bear genes encoding resistance to aminoglycosides, tetracycline, sulfamethoxazole trimethoprim and fluoroquinolones (Paterson and Bonomo 2005; Cabrera and Rodriguez, 2009; Rodriguez-Bano *et al*., 2018). This significantly reduces the treatment options for diseases caused by ESBL- producing strains. Since the emergence of ESBL- producing *E. coli*, antibiotic treatment delay had been reported for clinical cases, which were eventually associated with high mortality rate of approximately 61 % (Melzer and Petersen, 2007). Although community-acquired ESBL-producing *E. coli* bacteraemic infection rate of 6.6% is lower than hospital-acquired bacteraemic infection rate of 26.8% its public health significance still remains (Melzer and Petersen, 2007).

In addition, 48% (12 of the 25 isolates) were found to be resistant or intermediate to the carbapenem imipenem. Eight of the 11 carbapenem resistant isolates tested (or 73%) were positive for carbapenemase production. This is alarming since carbapenems are often considered the antibiotics of last resort for multiple drug resistant Enterobacteriaceae (CDC, 2017), including ESBL-producing strains (Paterson and Bonomo 2005). It is reported that in the United States, almost half of hospital patients who get bloodstream infections from CRE bacteria die from the infection (Centers for Diseases Control and Prevention 2017).

Both ESBLs and carbapenemases are encoded by genes that are often carried in mobile genetic elements such as plasmids and integrons, which can be transferred from one microorganism to another, resulting to the conversion of susceptible strains to resistant ones (Paterson and Bonomo, 2005; Cabrera and Rodriguez 2009; Berglund, 2015: Shirani, 2016). Moreover, in addition to clinical environments where heavy usage of antimicrobials is a common practice, the significant role of nonclinical environments in the dissemination of antibiotic resistance genes have been emphasized. Berglund (2015) reported that horizontal gene transfer events are likely to be common in aquatic environments (such as in aquaculture ponds), and integrons are well suited for facilitating dissemination of the resistance genes in the environment. Although the administration of antimicrobial-supplemented feeds has been shown to result in higher yield in total aquaculture production, the large-scale use of antibiotics in aquaculture is believed to further contribute to the selection of antimicrobial resistant microorganisms to survive and be disseminated, resulting in an increase in the prevalence of drug resistant strains (Ciceron *et al*., 2008; Saptoka *et al*., 2008; Ryu *et al*., 2012). The use of a single antimicrobial in the feeds can effectively select for MDR strains if the particular resistance gene is found in genetic elements such as plasmids and transposons that also carry other drug resistance genes. Studies have also shown the transmission of MDR microorganisms from aquaculture products (Saptoka *et al.,* 2008) and from farm animals to man (Alexander *et al.* 2010) as a consequence of handling and through the food chains.

The 25 *E. coli* isolates in the present study exhibited 20 different resistance phenotypes, indicating that these are different strains, and are not clones of each other. The results showed the diversity of the *E. coli* isolates from Tilapia from the two wet markets that were procured from different vendors with different suppliers at different times, suggesting the rampant presence of MDR strains in the

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

different aquaculture fish ponds from which these were taken.

CONCLUSION

Results of the study showed a high prevalence of MDR *E. coli* from the gills and intestines of *O. niloticus*. In addition, there was high prevalence of ESBL and carbapenemase-producing strains among the MDR isolates. The public health significance of the results in the present study cannot be overemphasized, if we were to consider the transmission of MDR strains and their resistance genes among the handlers, the consuming public and the environment. The results of the study should serve as a wakeup call for the government agencies concerned to continuously monitor the prevalence of MDR strains, and to evaluate the routine use of antimicrobials in aquaculture, as this selects for antimicrobial resistant microbial isolates in the animal flora and their surroundings to survive and be disseminated.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Molecular Science Unit Laboratory of the Center for Natural Science and Environmental Research of the De La Salle University Manila for the use of the laboratory facilities. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare no conflict of interest and ethical approval is not required.

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538