Brief communication

Mossambicus tilapia (*Oreochromis mossambicus*) collected from water bodies impacted by urban waste carries extended-spectrum beta-lactamases and integron-bearing gut bacteria

NACHIKET P MARATHE[1,†,‡], SWAPNIL S GAIKWAD[1,2,†], ANKITA A VAISHAMPAYAN[1], MANDAR H RASANE[1], YOGESH S SHOUCHE[1] and WASUDEV N GADE[2,*]

[1]*Microbial Culture Collection, National Centre for Cell Science, Pune 411 007, India*

[2]*Department of Biotechnology, Savitribai Phule Pune University, Pune 411 007, India*

[†]*Equal contribution.*

[‡]*Current address: Department of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy at the University of Gothenburg, SE-413 46, Göteborg, Sweden*

**Corresponding author (Email, wngade@unipune.ac.in)*

Oreochromis mossambicus (Peters 1852) (Tilapia) is one of the most consumed fish globally. Tilapia thrives well in environments polluted by urban waste, which invariably contain antibiotic-resistant bacteria and antibiotic resistance genes (ARGs). Thus, Tilapia surviving in such polluted environments may serve as a potential source for dissemination of ARGs. To investigate this, we isolated bacterial strains from gut of Tilapia found in polluted rivers and lakes near Pune, India, and studied the prevalence of resistance genes by molecular methods. A total of 91 bacterial strains were obtained, which include fish pathogens and human pathogens such as *Aeromonas hydrophila*, *Klebsiella pneumoniae, E. coli, Serratia marcescens, Enterobacter* spp. and *Shigella* spp. Overall the prevalence of class 1 integrons, class 2 integrons, extended-spectrum beta-lactamases (ESBLs) $bla_{\text{CTX-M}}$, bla_{SHV} and *aac(6')-Ib-cr* gene was 38%, 24%, 38%, 31% and 31% respectively. Forty-two percent of the *Enterobacteriaceae* strains carried $bla_{\text{CTX-M}}$ gene, which is a common ESBL gene in clinics. The study demonstrates that tilapia found in the polluted waters can serve as reservoirs and an alternative route for human exposure to clinically important ARG-carrying bacteria. The consumption and handling of these fish may pose a potential health risk.

[Marathe NP, Gaikwad SS, Vaishampayan AA, Rasane MH, Shouche YS and Gade WN 2016 Mossambicus tilapia (*Oreochromis mossambicus*) collected from water bodies impacted by urban waste carries extended-spectrum beta-lactamases and integron-bearing gut bacteria. *J. Biosci.* **41** 341–346]

1. Introduction

Tilapia is the most commonly found Cichlid fish worldwide (Singh and Lakra 2011). Tilapia is known to tolerate different temperature ranges, poor quality water, and low dissolved oxygen. Tilapia is also known to survive in the polluted environments where most of the other fish fail to survive (Huang *et al.* 2003; Wu and Hwang 2003; Perez *et al.* 2006; Jayaseelan *et al.* 2014). Tilapia from polluted waters is still consumed in India, due to its abundant availability and lower cost compared with other fish (Canonico *et al.* 2005).

Anthropogenic activities like urban waste, antibiotic production waste and animal farming increase the abundance of antibiotic-resistant bacteria and antibiotic resistance genes (ARGs) in the receiving waters and sediments (Kümmerer

Keywords. Antibiotic resistance; ESBLs; integrin; Mossambicus tilapia

Supplementary materials pertaining to this article are available on the Journal of Biosciences Website.

DOI: 10.1007/s12038-016-9620-2

Published online: 29 June 2016

2009; Marathe *et al.* 2013). The presence of Tilapia in such polluted environments exposes it to many potential bacterial pathogens carrying ARGs. Studies have shown that clinically important genes, such as extended-spectrum beta-lactamases (ESBLs) and integrons, increase in abundance in the receiving rivers, downstream of the waste water treatment plants (Lu *et al.* 2010; Kristiansson *et al.* 2011). ESBLs confer resistance to a wide variety of beta-lactam antibiotics. Hence, ESBL-carrying pathogens pose a serious threat for treating infections (Pagani *et al.* 2003; Naseer and Sundsfjord 2011). Integrons are mobile genetic elements responsible for integration and expression of gene cassettes. They are often associated with ARGs and considered as markers of horizontal gene transfer potential of a bacterial strain (Mazel 2006; Gillings 2014). ESBLs (Abgottspon *et al.* 2014) and integron (Schmidt *et al.* 2001) are reported in bacterial strains isolated from cultured fish. The consumption of fish carrying such ARGs by humans and other predators such as birds would serve as a route for the spread of ARGs through contact and/or food webs. Although there are reports on ARGs in bacteria associated with farmed Tilapia in Malyasia and Trinidad (Newaj-Fyzul *et al.* 2008; Budiati *et al.* 2013), there is no comprehensive study on the prevalence of ARGs associated with the gut bacteria of Tilapia thriving in waters polluted by urban waste, especially from India.

The aim of the current study was to isolate bacterial strains from the gut of Tilapia collected from polluted water bodies near Pune, India, and characterize these strains for the presence of resistance factors such as class 1, class 2 integrons, ESBLs – $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{OXA} and *aac(6')-Ib-cr* genes – using molecular methods. This would provide insights into potential of Tilapia as a dissemination route for ARGs and pathogens in the environment.

2. Material and methods

2.1 *Collection and identification of fish*

The samples were collected from Mula river, which is one of the rivers that flows through Pune city and is highly polluted due to industrial and sewage pollution (*www.cpcb.nic.in*). Samples were also collected from a lake near Pune (Talegaon Dabhade), which is known to be polluted by urban waste. Lakes and rivers represent different habitat, the latter being dynamic. Studies have shown that the fish gut microbiota is significantly influenced by the habitat type (Smith *et al.* 2015). Hence, in order to understand the differences in ARGs abundances in fish gut flora from these polluted ecosystems, we collected fish from both river and lake ecosystems. Fish were collected from three different sites of the river spanning a stretch of 0.2 km at each site, and 0.1 km from lake with the help from local fishermen. Water samples were collected at each site and physiochemical parameters of water were studied (table 1). Fish (3-4 individuals from each site) were transported to the laboratory and processed further on the same day.

Species identification of fish was done using DNA barcoding. Briefly, DNA was isolated from the gills of the fish using DNeasy Blood and Tissue Kit (Qiagen, USA) according to the manufacturer's protocol. *Cytochrome oxidase I* (COI) gene was amplified and sequenced using the method described earlier (Gaikwad *et al.* 2012). For bacterial isolation, fish were surface sterilized with 70% ethanol. Fish were dissected aseptically and gut content of the fish (three individuals) was homogenized in 10 mL of sterile phosphate-buffered saline (PBS), pH 7.2 (Nawaz *et al.* 2010).

2.2 *Isolation of bacterial strains*

Bacterial strains were isolated by the spread plate method. Briefly, 1.0 mL of gut homogenate was suspended in 9.0 mL of sterile PBS (pH 7.2). The serial dilutions were plated onto Tryptic soy agar (TSA) and R2A agar media and incubated at 30°C, for 24 to 48 h. Isolated colonies of different morphologies were selected and re-streaked to obtain pure cultures. The strains were further stored as glycerol stocks at −70°C.

2.3 *DNA extraction, PCR and DNA sequencing*

Genomic DNA was extracted from freshly grown pure cultures using DNeasy Blood and Tissue Kit (Qiagen, USA) according to the manufacturer's protocol.The isolated strains were identified by 16S rRNA gene sequencing as described earlier (Marathe *et al.* 2013) using universal primer set 27F and 1488R (table 2). The PCR reaction constituted 1X standard Taq Buffer, 200 nM dNTPs, 0.4 µM of each primer, 0.625 U Taq polymerase (Invitrogen, USA). All PCR reactions were performed for 35 cycles in 25µl volume. The PCR products were analyzed on 1% agarose gel and stained using SYBR Safe DNA gel stain (Invitrogen, USA). The purified PCR products were sequenced using BigDye Terminator Cycle Sequencing Ready Reaction Kit v 3.1 in an automated 3730xl DNA analyzer (Applied Biosystems Inc, USA). The detection of antibiotic resistance genes was done by PCR using primers listed in table 2. The isolates positive for ESBLs were grown on TSA plates containing 100 µg/mL ampicillin to confirm the expression of beta-lactamase gene.

3. Results and discussion

3.1 *Identification of fish and bacterial strains*

DNA barcoding analysis showed that all the fish collected in this study belonged to *Oreochromis mossambicus*. The

Table 1. Details of the sampling sites and chemical parameters of the water samples

Sample number	Sample code	Habitat Type	Collection site	Collection date	Co-ordinates	pH	TDS (mg/L)	Conductivity (mS/cm)	DO (mg/L)	Nitrite (mg/L)
1	TIL_TAL	Lake	Talegaon Dabhade	16-10-2013	18.724451 N, 73.665914 E	7.4	155	300	5	9
2	TIL_CHAN	River	Chande	15-10-2013	18.554903 N, 73.713914 E	7.4	130	270	5.8	10
3	TIL_WAK	River	Wakad	15-10-2013	18.588581 N, 73.762082 E	7.7	230	480	3	21
4	TIL_AUN	River	Aundh	15-10-2013	18.568315 N, 73.807678 E	7.8	260	530	2.3	14

sequences for *COI* gene are submitted to GenBank under accession number KU170710 to KU170713.

A total of 91 bacterial strains were obtained from the four samples. All the strains were identified based on 16S rRNA gene sequence. These sequences are submitted to GenBank under accession number KT998807 to KT998893 and KU163141 to KU163144. The strains were dominated by family *Aeromonadaceae* (35%) followed by *Enterobacteriaceae* (34%). At genus level, *Aeromonas* was the most prevalent genera followed by *Bacillus* and *Enterobacter* (supplementary table 1).

Along with fish pathogens (*Aeromonas hydrophila, Plesiomonas shigelloides),* we identified many potential human pathogens (*Klebsiella pneumoniae, E. coli, Serratia marcescens, Enterobacter sp.* and *Shigella sp.)* from Tilapia gut. Emerging opportunistic human pathogens such as *Leclercia adecarboxylata* and *Staphylococcus sciuri* were also detected (Brenden *et al.* 1988; Stepanović *et al.* 2001; Stock *et al.* 2004). Fish pathogen, *Plesiomonas shigelloides* is known to cause gastroenteritis and sometimes other infections in humans (Brenden *et al.* 1988). The presence of these pathogenic bacteria in the Tilapia gut is thus a cause of concern, as it can serve as a reservoir of potential human pathogens in the environment.

3.2 *The prevalence of resistance factors*

Screening of these bacterial isolates for the presence of integrons showed that overall 38% (35/91) of the isolates carried class 1 integrons, while 24% carried class 2 integrons (figure 1). Furthermore, ten percent of the isolates carried both class 1 integron and class 2 integrons. Previous studies on *Aeromonas* sp. isolated from *Cyprinus carpio* from Mexico and Australia showed 43% and 31% of prevalence of class 1 integron, respectively (Ndi and Barton 2011; Sarria-Guzman *et al.* 2014). Our findings are comparable with these reports; we detected class 1 integrons in 38% of the *Aeromonas* sp. isolates. However, considerably higher prevalence of class 1 integrons (48%) and other resistance like $bla_{\text{CTX-M}}$ (42%) genes were observed in *Enterobacteriaceae* isolates. Class 2 integrons were detected in the present study with highest prevalence in *Aeromonas sp.* (31%), followed by *Bacillus* sp. (19%). Both class 1 and class 2 are known to be associated with different antibiotic resistance genes and considered as markers of horizontal gene transfer potential of a bacterial strain (Mazel 2006; Gillings 2014). Presence of integrons in the bacterial strains isolated from Tilapia gut suggests that these strains have high potential for horizontal gene transfer.

Expression of ESBL enzymes by pathogenic bacteria is a serious clinical problem. In the present study, highest prevalence was observed for $bla_{\text{CTX-M}}$ (38%) followed by bla_{SHV} (31%) and bla_{OXA} (7%) in bacterial isolates from Tilapia gut. *Enterobacteriaceae* had slightly higher prevalence of $bla_{\text{CTX-M}}$ (42%). All the ESBL-carrying strains showed

Table 2. The list of primers used in the study

Primer	Sequence	Description	
27F	CCAGAGTTTGATCMTGGCTCAG	16S rRNA gene forward	(Marathe *et al.* 2013)
1488R	CGGTTACCTTGTTACGACTTCACC	16S rRNA gene reverse	
Int1F	GGGTCAAGGATCTGGATTTCG	Class 1 integrase forward	(Mazel et al. 2000)
Int1R	ACATGGGTGTAAATCATCGTC	Class 1 integrase reverse	
Int2F	CACGGATATGCGACAAAAAGGT	Class 2 integrase forward	(Mazel et al. 2000)
Int2R	GTAGCAAACGAGTGACGAAATG	Class 2 integrase reverse	
OXAF	ATGAAAAACACAATACATATC	ESBL OXA forward	(Mendonca *et al.* 2007)
OXAR	AATTTAGTGTGTTTAGAATGG	ESBL OXA reverse	
CTX-MF	ATGTGCAGYACCAGTAARGT	ESBL CTX-M forward	(Pagani *et al.* 2003)
CTX-MR	TGGGTRAARTARGTSACCAGA	ESBL CTX-M reverse	
SHVF	AGGATTGACTGCCTTTTTG	ESBL SHV forward	(Colom *et al.* 2003)
SHVR	ATTTGCTGATTTCGCTCG	ESBL SHV reverse	
AAC6F	TTGCGATGCTCTATGAGTGGCTA	*aac(6')-Ib-cr* forward	(Park *et al.* 2006)
AAC6R	TGCTCTATGAGTGGCTA	*aac(6')-Ib-cr* reverse	

growth on TSA plates containing 100 µg/mL ampicillin, thus confirming the production of a beta-lactamase by these strains. This illustrates that Tilapia serves as important reservoir for bla_{CTX-M}-carrying bacteria in the environment. ESBL-carrying *Enterobacteriaceae* pose a major threat for treatment in clinics, especially particularly those carrying CTX-M-type ESBL (Naseer and Sundsfjord 2011). Studies have shown that the bla_{CTX-M} gene in pathogens is often associated with different mobile genetic elements, one of them being integrons (Naseer and Sundsfjord 2011). In the present study, we detected integrons in 74% of bla_{CTX-M}-carrying isolates. Thus, implicating possible high tendencies for participation in horizontal gene transfer events of these bla_{CTX-M}-gene-carrying strains. Along with ESBLs, *aac(6')-Ib-cr* gene, which confers resistance to aminoglycosides and a low level resistance to ciprofloxacin, was detected in 31% of the isolates. The *aac(6')-Ib-cr* gene is usually associated with mobile genetic elements like conjugative plasmids, transposons or integrons (Park *et al.* 2006; Yang *et al.* 2008). Thus, the presence of potential human pathogens carrying ESBLs and other resistance factors known to be associated with mobile genetic elements suggest that Tilapia may acts as important reservoir of clinically relevant resistance factors and pathogens in the environment.

3.3 *Prevalence of resistance factors in fish from lake and river ecosystems*

The gut bacteria of Tilapia caught from lake had considerably higher abundance of resistance genes compared with those from rivers (figure 2). Previous studies have shown that gut flora of fish varies according to habitat type (Smith *et al.*

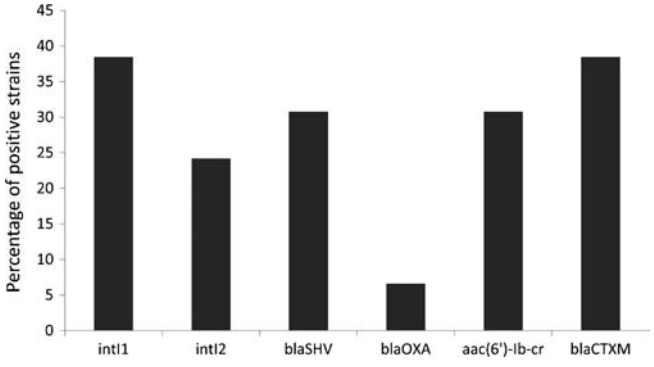

Figure 1. Prevalence of resistance genes among the bacterial strains isolated from Mossambicus tilapia gut. *IntI1*, class 1 integrase; *IntI2*, class 2 integrase; bla_{SHV}, ESBL SHV type; bla_{OXA}, ESBL OXA type; bla_{CTX-M}, ESBL CTXM type; *aac(6')-Ib-cr,* aminoglycoside acetyltransferase.

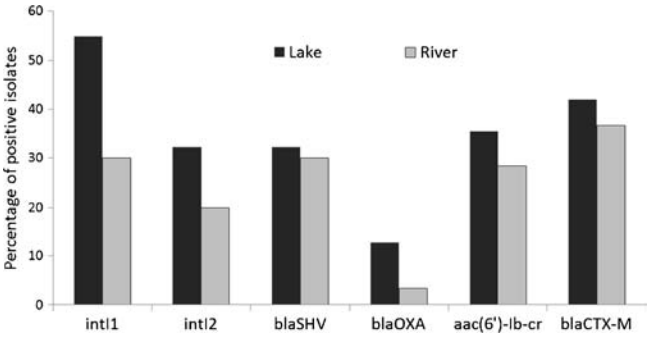

Figure 2. Differences in resistance genes prevalence among the bacterial strains isolated from Mossambicus tilapia from Lake and River. *IntI1*, class 1 integrase; *IntI2*, class 2 integrase; bla_{SHV}, ESBL SHV type; bla_{OXA}, ESBL OXA type; bla_{CTX-M}, ESBL CTXM type; *aac(6')-Ib-cr,* aminoglycoside acetyltransferase.

2015). The differences in ARG carriage by gut flora of fish from these two different ecosystems may be attributed to the load of ARGs in the environment. A lake is a static water body; thus, pollution with sewage would have a long-term effect on the fauna of lake as compared with a river. A river is dynamic ecosystem with flowing water and changing conditions. Although this is true, we have not studied the abundance of ARGs and bacterial flora of water from where fish were caught. This would have given important insights about the role of environment in shaping the ARGs' abundance in fish gut flora. To understand this, we plan a broader follow-up study with larger number of samples and sampling sites including the uncontaminated sites, targeting ARGs abundance in water samples and bacterial flora of water, along with Tilapia gut bacteria. Nevertheless, the current study for the first time shows that Tilapia thriving in polluted water bodies carry bacterial pathogens bearing clinically important ARGs.

Environmental bacteria bearing resistance genes can pose a potential public health risk. The bacteria from polluted waters can be acquired by humans in many ways such as contact with the water or consumption of plants irrigated with contaminated water (Wellington *et al.* 2013). Our study demonstrates that Mossambicus tilapia, which is popularly used as a food source, carries gut bacteria bearing clinically important genes. Thus, it might serve as a vector for transmission of resistance genes to human commensal and pathogens. Although in India, fish are properly cleaned before cooking, handling of fish and poor disposal of non-edible parts potentially allow ARGs to disseminate in the food web. These fish (dead or alive) and their remains are often consumed by the stray dogs and birds, which also serve as another route for the dissemination of ARGs in the environment.

4. Conclusion

To the best of our knowledge this is the first study addressing the prevalence of resistance factors in gut bacteria of Tilapia dwelling in water bodies impacted with urban waste from India. This study demonstrates that fish found in polluted waters serve as a reservoir of clinically important antibiotic resistance genes and may serve as an alternative route for their dissemination to humans. Furthermore, Tilapia is known for local migration. Thus, it might disseminate resistance-gene-carrying bacteria to larger parts of the river during migration. Proper management of urban waste and awareness on the consumption of fish from polluted environments should be promoted in order to reduce the spread of antibiotic resistance through fish thriving in polluted waters.

Acknowledgements

This work was supported by the Dr DS Kothari Postdoctoral Fellowship Scheme by University Grant Commission, India, to SSG; Departmental Research and Development Grant from the Department of Biotechnology,India, and University for Potential Excellence (UPE-II) Phase Program and DBT, India, for providing funds to carry out this work under the MCC project grant no. BT/PR10054/NDB/52/94/2007.

References

Abgottspon H, Nuesch-Inderbinden MT, Zurfluch K, Althaus D, Hachler H and Stephan R 2014 Enterobacteriaceae with extended-spectrum- and pAmpC-Type -lactamase-encoding genes isolated from freshwater fish from two lakes in Switzerland. *Antimicrob. Agents Chemother.* **58** 2482–2484

Brenden RA, Miller M and AJanda JM 1988 Clinical disease spectrum and pathogenic factors associated with *Plesiomonas shigelloides* infections in humans. *Rev. Infect. Dis.* **10** 303–316

Budiati T, Rusul G, Wan-Abdullah WN, Arip YM, Ahmad R and Thong KL 2013 Prevalence, antibiotic resistance and plasmid profiling of *Salmonella* in catfish (Clarias gariepinus) and tilapia (Tilapia mossambica) obtained from wet markets and ponds in Malaysia. *Aquaculture* **372** 127–132

Canonico GC, Arthington A, McCrary JK and Thieme ML 2005 The effects of introduced tilapias on native biodiversity. *Aquat. Conserv. Mar. Freshwat. Ecosyst.* **15** 463–483

Colom K, Pacrez J, Alonso R, Fernández-Aranguiz A, Lariño E and Cisterna R 2003 Simple and reliable multiplex PCR assay for detection of blaTEM, blaSHV and blaOXA-1 genes in Enterobacteriaceae. *FEMS Microbiol. Lett.* **223** 147–151

Gaikwad SS, Ghate HV, Ghaskadbi SS, Patole MS and Shouche YS 2012 DNA barcoding of nymphalid butterflies (Nymphalidae: Lepidoptera) from Western Ghats of India. *Mol. Biol. Rep.* **39** 2375–2383

Gillings MR 2014 Integrons: past, present, and future. *Microbiol. Mol. Biol. Rev.* **78** 257–277

Huang YK, Lin KH, Chen HW 2003 Arsenic species contents at aquaculture farm and in farmed mouthbreeder (Oreochromis mossambicus) in blackfoot disease hyper endemic areas. *Food Chem Toxicol* **41** 1491–1500

Jayaseelan C, Rahuman A and ARamkumar R 2014 Ecotoxicology and environmental safety effect of sub-acute exposure to nickel nanoparticles on oxidative stress and histopathological changes in Mozambique tilapia *Oreochromis mossambicus. Ecotoxicol Environ Saf.* **107** 220–228

Kristiansson E, Fick J, Janzon A, Grabic R, Rutgersson C, Weijdegård B, Söderström H and Larsson DG 2011 Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. *PLoS One* **6** 17038

Kümmerer K 2009 Antibiotics in the aquatic environment – a review – part I. *Chemosphere.* **75** 417–434

Lu SY, Zhang YL, Geng SN, Li TY, Ye ZM, Zhang DS, Zou F and Zhou HW 2010 High diversity of extended-spectrum beta-lactamase-producing bacteria in an urban river sediment habitat. *Appl. Environ. Microbiol.* **76** 5972–5976

Marathe NP, Regina VR, Walujkar SA, Charan SS, Moore ERB, Larsson DGJ and Shouche YS 2013 A treatment plant receiving waste water from multiple bulk drug manufacturers is a reservoir

for highly multi-drug resistant integron-bearing bacteria. *PLoS One* **8** 77310

Mazel D 2006 Integrons: agents of bacterial evolution. *Nat. Rev. Microbiol.* **4**(8):608–620

Mazel D, Dychinco B, Webb VA and Davies J 2000 Antibiotic resistance in the ECOR collection: integrons and identification of a novel aad gene. *Antimicrob. Agents Chemother.* **44** 1568–1574

Mendonca N, Leitao J, Manageiro V, Ferreira E and Canica M 2007 Spread of extended-spectrum-lactamase CTX-M-Producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. *Antimicrob. Agents Chemother.* **51** 1946–1955

Naseer U and Sundsfjord A 2011 The CTX-M conundrum: dissemination of plasmids and Escherichia coli clones. *Microb. Drug Resist.* **17** 83–97

Nawaz M, Khan SA, Khan AA and Sung K 2010 Detection and characterization of virulence genes and integrons in *Aeromonas veronii* isolated from catfish. *Food Microbiol.* **27** 327–331

Ndi OL and Barton MD 2011 Incidence of class 1 integron and other antibiotic resistance determinants in Aeromonas spp. from rainbow trout farms in Australia. *J. Fish Dis.* **34** 589–599

Newaj-Fyzul A, Mutani A, Ramsubhag A and Adesiyun A 2008 Prevalence of bacterial pathogens and their anti-microbial resistance in tilapia and their pond water in Trinidad. *Zoonoses Public Health* **55** 206–213

Pagani L, Dell'Amico E, Migliavacca R, D'Andrea MM, Giacobone E, Amicosante G, Romero E and Rossolini GM 2003 Multiple CTX-M-type extended-spectrum -lactamases in nosocomial isolates of *Enterobacteriaceae* from a hospital in Northern Italy. *J. Clin. Microbiol.* **41** 4264–4269

Park CH, Robicsek A, Jacoby GA, Sahm D and Hooper DC 2006 Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. *Antimicrob. Agents Chemother.* **50** 3953–3955

Perez JE, Nirchio M, Alfonsi C and Munoz C 2006 The biology of invasions : the genetic adaptation paradox. *Biol. Invasions* **8** 1115–1121

Sarria-Guzman Y, Lopez-Ramirez MP, Chavez-Romero Y, Ruiz-Romero E, Dendooven L and Bello-Lopez JM 2014 Identification of antibiotic resistance cassettes in class 1 integrons in Aeromonas spp. strains isolated from fresh fish (Cyprinuscarpio L.). *Curr. Microbiol.* **68** 581–586

Schmidt AS, Bruun MS, Dalsgaard I and Larsen JL 2001 Incidence, distribution, and spread of tetracycline resistance determinants and integron-associated antibiotic resistance genes among motile aeromonads from a fish farming environment. *Appl. Environ. Microbiol.* **67** 5675–5682

Singh AK and Lakra WS 2011 Risk and benefit assessment of alien fish species of the aquaculture and aquarium trade into India. *Rev. Aquac.* **3** 3–18

Smith CC, Snowberg LK, Caporaso JG, Knight R and Bolnick DI 2015 Dietary input of microbes and host genetic variation shape among-population differences in stickleback gut microbiota. *ISME J.* **9** 2515–2526

Stepanović S, Vuković D, Trajković V, Samardžić T, Ćupić M and Švabić-Vlahović M 2001 Possible virulence factors of *Staphylococcus sciuri. FEMS Microbiol. Lett.* **199** 47–53

Stock I, Burak S and Wiedemann B 2004 Natural antimicrobial susceptibility patterns and biochemical profiles of *Leclerciaa decarboxylata* strains. *Clin. Microbiol. Infect.* **10** 724–733

Wellington EMH, Boxall ABA, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, *et al*. 2013 The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect. Dis.* **13** 155–165

Wu SM and Hwang PP 2003 Copper or Cadmium pretreatment increases the protection against cadmium toxicity in Tilapia larvae (Oreochromis mossambicus). *Zool. Stud.* **42** 179–185

Yang H, Chen H, Yang Q, Chen M and Wang H 2008 High prevalence of plasmid-mediated quinolone resistance genes qnr and aac(6')-Ib-cr in clinical isolates of Enterobacteriaceae from nine teaching hospitals in China. *Antimicrob. Agents Chemother.* **52** 4268–4273

MS received 22 January 2016; accepted 19 May 2016

Corresponding editor: Gagandeep Kang