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# Research Article Characterization of Quinolone Resistant Determinants in *Morganella morganii* Isolated from Pet Turtles

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# Abstract

**Background and Objective:** Drug resistance in bacteria is a challenge both in human and veterinary medicine. This study was conducted to characterize quinolone resistant determinants in *Morganella morganii* isolated from pet turtles. **Materials and Methods:** Antimicrobial susceptibility of twenty-two *M. morganii* isolates against nalidixic acid, ciprofloxacin, ofloxacin and levofloxacin was examined by disk diffusion assay and the Minimum Inhibitory Concentration (MIC). Substitutions of the Quinolone Resistance Determining Region (QRDR) and Plasmid Mediated Quinolone Resistance (PMQR) genes were detected using conventional PCR assays and sequencing. **Results:** Three isolates were resistant to the all tested quinolones and one isolate was resistant only to nalidixic acid. In QRDR substitution in *parC*. Two isolates displayed the Ser463Ala, Ser464Tyr and novel Glu466Asp substitutions in *gyrB* and the Ser80lle substitution in *parC*. Two isolates. The *aac-(6')-lb-cr* gene variant was identified in 50% of the isolates. In addition, neighbor-joining phylogenetic tree derived using *gyrB* gene sequences exhibited two distinct clads comprising, first; present study isolates with a quinolone-resistant isolate of human clinical origin and second; isolates of environmental origin. **Conclusions:** All results suggest healthy pet turtles might serve as a potential reservoir for quinolone-resistant *M. morganii* due to the high prevalence of PMQR determinants, especially, *qnrD* and target gene alterations in QRDR together with a novel mutation in *gyrB*.

Key words: Morganella morganii, pet turtle, QRDR mutations, PMQR genes, qnrD

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

# INTRODUCTION

*Morganella morganii* is a Gram-negative bacteria commonly found in the environment and in the intestinal tract of humans, mammals and reptiles as a part of the normal microflora<sup>1,2</sup>. It can involve in various infections including pneumonia, peritonitis, empyema, pericarditis, arthropathy, endophthalmitis, meningitis and wound infections in amphibians and reptiles<sup>3,4</sup>. It has been recorded that the studies related to *M. morganii* isolated from turtles are still scanty<sup>5</sup>. Meanwhile, the interest in wild animals as pets has increased over the past few years due to human curiosity and eccentricity. Several species of reptiles are bred as pets nowadays, especially exotic pet turtles have gained popularity in developed countries<sup>6,7</sup>.

However, pet turtles are well recognized as source of diverse pathogens and the direct and indirect contact associated contamination can transmit the pathogens to humans<sup>5</sup>. Over the years, many case studies have been conducted to assess the prevalence of *M. morganii* in human clinical cases, which usually caused nosocomial infections, particularly among the immunocompromised patients. *Morganella morganii* can lead to major clinical problems, such as wounds, urinary tract infections and septicaemia<sup>8,9</sup>. In a case study of *Morganella* infection, various groups of antibiotics including fluoroquinolones have been tested *in vitro* for the treatment<sup>2</sup>.

Quinolones are broad-spectrum antibiotics extensively used in human and veterinary medicine hence, has been resulted in rising levels of guinolone resistance. Morganella morganii from clinical and environmental sources have demonstrated increasing levels of guinolone resistance, which were frequently associated with the presence of resistance genes and related mechanisms<sup>9,10</sup>. In contrast, guinolone resistance mechanism is mediated by specific chromosomal mediated and Plasmid Mediated Quinolone Resistance (PMQR) genes. High level of guinolone resistance arises mainly due to chromosomally encoded mechanisms such as mutations in the Quinolone Resistance Determining Region (QRDR) of DNA gyrase and topoisomerase IV. The DNA gyrase is the primary target of guinolones, consisting of two subunits that are encoded by *qyrA* and *qyrB* genes. Topoisomerase IV is the secondary target of quinolones, which is also comprising of two subunits encoded by *parC* and parE genes<sup>11</sup>.

Plasmid Mediated Quinolone Resistance (PMQR) causatives include *qnr*-type pentapeptide proteins (*qnrA, qnrB, qnrC, qnrD* and *qnrS*) and *aac(6')-lb-cr* conferring low-level of resistance to fluoroquinolones compared to

chromosomally mediated resistance<sup>12,13</sup>. Prevalence of *qnr* genes could vary with the species of bacteria. Several past studies identified *qnrD* as more prevalent PMQR determinants in *M. morganii* isolates. The *qnrD* gene is a relatively uncommon PMQR gene, which has been detected in members of the Proteeae family from different origin<sup>14-16</sup>.

These resistance genes consisting plasmids and transposons are known as mobile genetic elements that can be transferred horizontally among distantly related lineages. Particularly, the water environment is more favorable for the transmission of resistant bacteria from animals to humans, thus, *M. morganii* as an opportunistic pathogen might be dangerous vector for the dissemination of antibiotic resistance genes through the aquatic environment<sup>9,11</sup>.

Therefore, the current study aimed to evaluate the quinolone resistance characteristics and related genetic background in pet turtle-borne *M. morganii* in order to advocate the awareness about the pathogen counting concerned to public health. This study also aimed to determine the quinolone susceptibility, occurrence of PMQR genes [*qnrA, qnrB, qnrC, qnrD, qnrS* and *aac(6')-lb-cr*] and chromosomal QRDR mutations in *gyrA, gyrB* and *parC* in pet turtle-associated *M. morganii*.

### **MATERIALS AND METHODS**

**Bacterial isolates:** Twenty-two isolates of *M. morganella* were obtained from fecal samples of 6 commercially available pet turtle species namely, Chinese stripe-necked turtles (*Ocadia sinensis*), yellow-bellied sliders (*Trachemys scripta scripta*), river cooters (*Pseudemys concinna concinna*), Northern Chinese softshell turtles (*Pelodiscus maackii*), African sideneck turtles (*Pelusios castaneus*) and common musk turtles (*Sternotherus odoratus*). Turtles were purchased randomly through pet shops in Korea and had an average weight of  $15\pm 2$  g, carapace diameter of  $40\pm 5$  mm and were under 4 weeks of age. All turtles did not show any clinical signs of disease and confirmed not to have a treatment history hence, considered as healthy.

**Bacterial identification using 16S rRNA gene amplification and sequencing:** Genomic DNA was extracted from presumptively identified *M. morganii* isolates by Chelex 100 extraction method and PCR for 16S rRNA was performed using universal primers; 12F and 1492R. Amplicons were sequenced and tested for the similarity using BLAST algorithm of NCBI database so as to confirm the species status. Determination of quinolone susceptibility and minimum inhibitory concentrations: Antibiotic susceptibility to nalidixic acid, ciprofloxacin, ofloxacin and levofloxacin was determined by disc diffusion on Mueller Hinton agar (MBcell Ltd., Seoul, Korea) using OXOID<sup>™</sup> antibiotic disks (Oxoid Co. Ltd., Seoul, Korea). Testing was confirmed by duplicating and the resistance profiles (resistant, intermediate, or susceptible) were assigned using criteria described by Clinical and Laboratory Standards Institute (CLSI)<sup>17</sup>. The Minimum Inhibitory Concentrations (MIC) were determined using broth microdilution method containing nalidixic acid (1-512 µg mL<sup>-1</sup>), ciprofloxacin, ofloxacin and levofloxacin (0.06-32 µg mL<sup>-1</sup>). Resistance breakpoints (resistant, intermediate, or susceptible) were assigned using criteria described by CLSI<sup>17</sup>.

Detection of mutations in guinolone resistancedetermining region (QRDR): The QRDRs of all isolates were examined by amplifying and sequencing gyrA (441 bp), qvrB (300 bp) and parC (204 bp) genes using primers (Table 1) described by Lascols et al.<sup>18</sup>. The PCR amplifications were conducted in 50 µL volumes consisting of 20 µL of Quick Taq<sup>®</sup> HS DyeMix (TOYOBO, Japan), 1  $\mu$ L of 20 pmol  $\mu$ L<sup>-1</sup> each primer and 2  $\mu$ L of the template under standard conditions. The PCR products were analyzed by electrophoresis on 2% (w/v) agarose gels. Amplimers were purified using Expin™ PCR SV kit (GeneAll®, Korea) and sequenced by Cosmogenetech Co. Ltd, Daejeon, Korea. The gyrA, gyrB and *parC* nucleotide sequences obtained from the present study were aligned and compared with published reference sequences using Mutation Surveyor V5.0.1 (SoftGenetics LLC, USA) in order to identify amino acid substitutions.

**Detection of quinolone resistance genes:** Total DNA (2  $\mu$ L) was subjected to PCR for amplifications of *qnrA*, *qnrB*, *qnrS*, *qnrC* and *qnrD* genes using primers (Table 1) described by Adachi *et al.*<sup>19</sup> and Dasgupta *et al.*<sup>20</sup>. The DNA fragments were detected by electrophoresis in a 2% (w/v) agarose gels. The presence of the *aac(6')-lb-cr* gene was investigated by PCR using primers (Table 1) described by Park *et al.*<sup>21</sup> and the presence of *cr* variant was investigated by sequencing and blasting in NCBI database.

Phylogenetic comparison of gyrB sequences with published

**sequences:** The sequences derived for *gyrB* gene region were analyzed and a neighbor-joining phylogenetic tree was obtained with 1000 bootstrap replications. For the analysis, four previously published *gyrB* sequences of *M. morganii* 

Table 1: Primers used for amplification of quinolone resistance genes

Genes	Primers	Sequences (5'-3')	Size (bp)
gyrA	GyrA1s-F	CTGCGCGGCTGTGTTATAATT	500
	GyrA2as-R	CCGTGCCGTCATAGTTATCAA	
gyrB	GyrB5s-F	CTGCCGGGCAAACTGGCAGA	300
	GyrB6-R	TCGACGTCCGCATCGGTCAT	
parC	TP1-Ecl-F	AAGAAATCCGCCCGTACCGT	150
	TP2-Ecl-R	CGGTGCCCCCAGTTCCCCT	
qnrA	QnrAm-F	AGAGGATTTCTCACGCCAGG	580
	QnrAm-R	TGCCAGGCACAGATCTTGAC	
qnrS	QnrSm-F	GCAAGTTCATTGAACAGGGT	428
	Qnrsm-R	TCTAAACCGTCGAGTTCGGCG	
qnrB	QnrB-F	GATCGTGAAAGCCAGAAAGG	496
	QnrB-R	ACGATGCCTGGTAGTTGTCC	
qnrC	qnrC-F	GGGTTGTACATTTATTGAATC	447
	qnrC-R	TCCACTITACGAGGTTCT	
qnrD	qnrD-F	CGAGATCAATTTACGGGGAATA	582
	qnrD-R	AACAAGCTGAAGCGCCTG	
aac(6')-lb	aac(6')-Ib-F	TTGCGATGCTCTATGAGTGGCTA	482
	aac(6')-lb-R	CTCGAATGCCTGGCGTGTTT	

Table 2: Details of the *gyrB* gene sequences of *M. morganii* downloaded from NCBI database for the phylogenetic analysis

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NCBI accession		Country of
numbers	Origin	record
AB972375.1	Waste water sediment isolate	India
DQ360899.1	Histamine-producing seafood isolate	Denmark
KF732712.1	Quinolone resistant human clinical isolate	Argentina
HM122057.1	M. morganii strain ATCC 25830	India

clinical and environmental isolates were obtained from the GenBank database (Table 2) and MEGA6 sequence analyzing software was used for aligning and construction of the phylogenetic tree.

#### RESULTS

**16S rRNA gene-based identification:** A subsequent BLAST search after 16S rRNA sequencing indicated a 99-100% match to *M. morganii* sequences available in GenBank which confirmed 22 isolates as *M. morganii*.

**Quinolone susceptibility and MICs of isolates:** Eighteen out of 22 (82%) isolates of *M. morganii* were susceptible to quinolones; nalidixic acid, ciprofloxacin, ofloxacin and levofloxacin both in disk diffusion test and MIC. Only 3 isolates were resistant to all quinolones tested in this study while one isolate was resistant to only nalidixic acid. Nalidixic acid MICs of resistant strains ranged from  $32 \ge 256 \ \mu g \ mL^{-1}$ , while ofloxacin, ciprofloxacin and levofloxacin MICs of resistant strains were ranged 16-32  $\ \mu g \ mL^{-1}$  (Table 3).

**The** *gyrA, gyrB* and *parC* **QRDR substitution analysis:** Alterations in the *gyrB* QRDR were observed in 23% (5/22) of the isolates. Three quinolone-resistant isolates displayed the

NA30 CIP5 OFX5 LVF5 NA   1M 24(5) 30(5) 33(5) 4(5)   3M 25(5) 32(5) 32(5) 33(5) 4(6)   5M 25(5) 32(5) 28(5) 33(5) 4(7)   6M 28(5) 32(5) 28(5) 33(5) 4(7)   6M 28(5) 31(5) 29(5) 33(5) 2(7)   10M 28(5) 33(5) 29(5) 33(5) 2(7)   11M 29(5) 33(5) 28(5) 31(5) 2(7)   11M 23(5) 33(5) 28(5) 31(5) 2(7)   11M 23(5) 36(5) 31(5) <th>LVF5 33(5) 32(5) 32(5) 32(5) 32(5) 32(5) 32(5) 33(5) 3</th> <th>NA 4(S) 4(S) 4(S) 2(S) 2(S) 2(S) 2(S)</th> <th>CIP 0.06(S)</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th>	LVF5 33(5) 32(5) 32(5) 32(5) 32(5) 32(5) 32(5) 33(5) 3	NA 4(S) 4(S) 4(S) 2(S) 2(S) 2(S) 2(S)	CIP 0.06(S)						
IM 24(5) 34(5) 30(5) 33(5) 4(5)   3M 25(5) 32(5) 29(5) 33(5) 4(6)   5M 24(5) 32(5) 29(5) 32(5) 4(7)   6M 28(5) 31(5) 29(5) 32(5) 4(7)   8M 27(5) 33(5) 29(5) 34(5) 29(5)   10M 28(5) 34(5) 29(5) 34(5) 29(5)   11M 29(5) 33(5) 27(5) 30(5) 21(5)   12M 27(5) 33(5) 27(5) 30(5) 21(5)   13M 0 (R) 0 (R) 0 (R) 31(5) 21(5)   13M 27(5) 30(5) 28(5) 31(5) 21(5)   13M 0 (R) 0 (R) 0 (R) 8(R) 25   14M 23(5) 34(5) 28(5) 31(5) 21(5)   15M 28(5) 30(5) 34(5) 21(5) 21(5)   16M	33(S) 32(S) 32(S) 32(S) 34(S) 31(S) 31(S) 31(S) 31(S) 31(S) 31(S) 31(S)	4(S) 4(S) 4(S) 2(S) 2(S) 2(S)	0.06(S)	OFX	LVF	gyrB	parC	dnrD	aac(6)-lb
3M 25(S) 32(S) 29(S) 32(S) 4(S)   5M 24(S) 32(S) 29(S) 32(S) 4(S)   6M 28(S) 31(S) 29(S) 33(S) 24(S)   8M 27(S) 33(S) 29(S) 33(S) 27(S)   10M 28(S) 34(S) 23(S) 34(S) 27(S)   11M 29(S) 33(S) 27(S) 33(S) 27(S)   12M 27(S) 33(S) 27(S) 33(S) 27(S)   13M 0 (R) 0 (R) 0 (R) 33(S) 27(S) 27(S)   13M 27(S) 33(S) 27(S) 33(S) 27(S) 27(S)   13M 0 (R) 0 (R) 0 (R) 0 (R) 27(S) 27(S)   14M 23(S) 34(S) 28(S) 34(S) 27(S)   15M 28(S) 34(S) 28(S) 34(S) 27(S)   16M 0 (R) 0 (R) 0 (R) 37(S)	32(S) 32(S) 30(S) 33(S) 33(S) 33(S) 31(S) 8 (R) 31(S) 31(S)	4(S) 4(S) 2(S) 2(S) 2(S)		0.125(S)	0.06(S)	wt	wt		aac(6)-lb
5M 24(5) 32(5) 28(5) 32(5) 32(5) 4(5)   6M 28(5) 31(5) 29(5) 30(5) 20(5) 30(5) 20(5)   8M 27(5) 33(5) 29(5) 34(5) 30(5) 20(5)   10M 28(5) 34(5) 29(5) 34(5) 30(5) 20(5)   11M 29(5) 33(5) 27(5) 30(5) 31(5) 20(5)   12M 27(5) 30(5) 33(5) 27(5) 30(5) 20(5)   13M 0 (R) 0 (R) 0 (R) 0 (R) 31(5) 20(5)   14M 23(5) 30(5) 28(5) 31(5) 20(5)   15M 23(5) 30(5) 34(5) 20(5)   16M 0 (R) 0 (R) 0 (R) 8(R) 25   17M 28(5) 34(5) 28(5) 31(5) 20(5)   17M 28(5) 34(5) 28(5) 31(5) 20(5)   17M	32(S) 30(S) 34(S) 33(S) 33(S) 31(S) 31(S) 31(S) 31(S)	4(S) 2(S) 2(S) 2(S)	0.06(S)	0.125(S)	0.06(S)	wt	wt	dnrD	aac(6)-lb
6M 28(S) 31(S) 29(S) 30(S) 21(S)   8M 27(S) 33(S) 29(S) 33(S) 21(S)   10M 28(S) 34(S) 23(S) 34(S) 21(S)   11M 29(S) 33(S) 27(S) 33(S) 21(S)   11M 29(S) 33(S) 27(S) 33(S) 21(S)   12M 27(S) 33(S) 27(S) 33(S) 21(S)   13M 0 (R) 0 (R) 0 (R) 31(S) 21(S) 21(S)   13M 27(S) 33(S) 28(S) 31(S) 21(S) 21(S)   13M 21(R) 23(S) 32(S) 31(S) 21(S) 21(S)   15M 23(S) 34(S) 28(S) 34(S) 21(S) 21(S)   15M 23(S) 24(S) 34(S) 31(S) 21(S) 21(S)   15M 28(S) 34(S) 28(S) 31(S) 21(S) 21(S)   16M 28(S)<	30(S) 34(S) 33(S) 30(S) 31(S) 34(S) 31(S) 31(S)	2(S) 2(S) 2(S) 2(S)	0.06(S)	0.125(S)	0.06(S)	wt	wt	dnrD	aac(6)-lb
8M 27(5) 33(5) 29(5) 34(5) 34(5) 21(5)   10M 28(5) 34(5) 28(5) 34(5) 28(5) 34(5) 26(5)   11M 29(5) 33(5) 27(5) 30(5) 33(5) 27(5)   12M 27(5) 30(5) 28(5) 31(5) 26(5) 26(5)   13M 0 (R) 0 (R) 0 (R) 0 (R) 8(R) 25   14M 23(5) 35(5) 30(5) 34(5) 26   15M 23(5) 34(5) 28(5) 31(5) 26   15M 23(5) 34(5) 28(5) 31(5) 26   16M 0 (R) 0 (R) 0 (R) 8(R) 25   17M 28(5) 34(5) 28(5) 31(5) 26   17M 28(5) 34(5) 28(5) 31(5) 26   17M 28(5) 34(5) 28(5) 31(5) 26   17M 28(5) 34(5) <td>34(S) 33(S) 30(S) 31(S) 8 (R) 34(S) 31(S)</td> <td>2(S) 2(S) 2(S)</td> <td>0.06(S)</td> <td>0.06(S)</td> <td>0.06(S)</td> <td>wt</td> <td>wt</td> <td>ı</td> <td>aac(6)-lb</td>	34(S) 33(S) 30(S) 31(S) 8 (R) 34(S) 31(S)	2(S) 2(S) 2(S)	0.06(S)	0.06(S)	0.06(S)	wt	wt	ı	aac(6)-lb
10M 28(5) 34(5) 28(5) 33(5) 21(5)   11M 29(5) 33(5) 27(5) 30(5) 33(5) 27(5)   12M 27(5) 30(5) 28(5) 31(5) 27(5)   13M 0 (R) 0 (R) 0 (R) 0 (R) 27(5) 30(5) 21(5)   13M 0 (R) 0 (R) 0 (R) 0 (R) 8 (R) 25   14M 23(5) 35(5) 30(5) 34(5) 21(5) 21(5)   15M 28(5) 34(5) 28(5) 34(5) 21(5) 21(5)   16M 0 (R) 0 (R) 0 (R) 0 (R) 8 (R) 25   17M 28(5) 34(5) 28(5) 31(5) 21(5)   17M 28(5) 34(5) 28(5) 31(5) 25   17M 28(5) 32(5) 31(5) 25   18M 26(5) 33(5) 36(5) 40(5)   210M 25(5) 33(5)	33(S) 30(S) 31(S) 8 (R) 34(S) 31(S)	2(S) 2(S)	0.06(S)	0.125(S)	0.06(S)	wt	wt	qnrD	aac(6)-lb
11M 29(S) 33(S) 27(S) 30(S) 21(S)   12M 27(S) 30(S) 27(S) 30(S) 21(S)   13M 0 (R) 0 (R) 0 (R) 0 (R) 21(S) 21(S) 21(S)   13M 0 (R) 0 (R) 0 (R) 0 (R) 8 (R) 25   14M 23(S) 35(S) 30(S) 34(S) 34(S) 24   15M 28(S) 34(S) 34(S) 34(S) 44   16M 0 (R) 0 (R) 0 (R) 8 (R) 25   17M 28(S) 34(S) 28(S) 31(S) 24   17M 28(S) 34(S) 28(S) 31(S) 24   18M 26(S) 32(S) 29(S) 31(S) 24   19M 24(S) 33(S) 33(S) 36(S) 44   21M 25(S) 33(S) 36(S) 34   21M 25(S) 33(S) 36 34	30(S) 31(S) 8 (R) 34(S) 31(S)	2(S)	0.06(S)	0.06(S)	0.06(S)	wt	wt	ı	aac(6)-lb
12M 27(S) 30(S) 28(S) 31(S) 2(S)   13M 0 (R) 0 (R) 0 (R) 0 (R) 2(S) 31(S) 25   14M 23(S) 35(S) 35(S) 30(S) 34(S) 25   15M 28(S) 34(S) 34(S) 34(S) 26   16M 0 (R) 0 (R) 0 (R) 8(R) 25   16M 0 (R) 0 (R) 0 (R) 26(S) 31(S) 27   17M 28(S) 34(S) 28(S) 34(S) 26   17M 28(S) 34(S) 28(S) 31(S) 27   18M 26(S) 32(S) 29(S) 31(S) 26   19M 24(S) 35(S) 33(S) 36(S) 46   21M 25(S) 33(S) 36(S) 36   21M 25(S) 33(S) 36 36	31(S) 8 (R) 34(S) 31(S)		0.06(S)	0.06(S)	0.06(S)	wt	wt	·	aac(6)-lb
13M 0 (R) 0 (R) 0 (R) 8 (R) 25   14M 23(S) 35(S) 35(S) 30(S) 34(S) 24   15M 23(S) 35(S) 30(S) 34(S) 34(S) 47   15M 28(S) 34(S) 28(S) 34(S) 26 47   16M 0 (R) 0 (R) 0 (R) 0 (R) 26 27 27   17M 28(S) 34(S) 28(S) 34(S) 26 27   17M 28(S) 34(S) 28(S) 34(S) 27 27   18M 26(S) 32(S) 28(S) 31(S) 27 27   19M 24(S) 35(S) 33(S) 36(S) 47 27   21M 25(S) 33(S) 33(S) 36(S) 47 27   21M 25(S) 33(S) 33(S) 36(S) 47 27   21M 25(S) 33(S) 36(S) 36(S) 47	8 (R) 34(S) 31(S)	2(S)	0.06(S)	0.25(S)	0.06(S)	S463A	wt	dnrD	aac(6)-lb-cr
14M 23(S) 35(S) 30(S) 34(S) 34(S) 4(S)   15M 28(S) 34(S) 34(S) 34(S) 34(S) 4(S)   16M 0 (R) 0 (R) 0 (R) 0 (R) 28(S) 31(S) 25   17M 28(S) 34(S) 28(S) 34(S) 26 31(S) 26   17M 28(S) 34(S) 28(S) 34(S) 26 31(S) 26   18M 26(S) 32(S) 28(S) 31(S) 26 31(S) 27   19M 24(S) 35(S) 30(S) 35(S) 40   21M 25(S) 33(S) 36(S) 36 40   21M 25(S) 33(S) 36 36 40   21M 25(S) 33(S) 36 36 40	34(S) 31(S)	256 (R)	<u>&gt;</u> 32 (R)	<u>&gt;</u> 32 (R)	16 (R)	S463A, S464Y, E466D	S801	dnrD	aac(6)-lb-cr
15M 28(S) 34(S) 28(S) 31(S) 2(S)   16M 0 (R) 0 (R) 0 (R) 0 (R) 2(S) 4(C) 2(S)	31(S)	4(S)	0.125(S)	0.125(S)	0.06(S)	wt	wt	dnrD	aac(6)-lb-cr
16M 0 (R) 0 (R) 0 (R) 25   17M 28(S) 34(S) 28(S) 32(S) 4(S)   18M 26(S) 32(S) 28(S) 31(S) 2(S)   18M 26(S) 32(S) 29(S) 31(S) 2(S)   19M 24(S) 35(S) 30(S) 35(S) 4(S)   21M 25(S) 33(S) 33(S) 36(S) 4(S)   21M 25(S) 33(S) 33(S) 36(S) 4(S)	i,	2(S)	0.06(S)	0.125(S)	0.06(S)	wt	wt	ı	aac(6)-lb
17M 28(S) 34(S) 28(S) 32(S) 4(S)   18M 26(S) 32(S) 29(S) 31(S) 2(S)   18M 26(S) 32(S) 29(S) 31(S) 2(S)   19M 24(S) 35(S) 30(S) 35(S) 4(S)   21M 25(S) 35(S) 33(S) 36(S) 4(S)   21M 25(S) 33(S) 33(S) 36(S) 4(S)	8 (K)	256 (R)	<u>&gt;</u> 32 (R)	<u>&gt;</u> 32 (R)	16 (R)	S463A, S464Y, E466D	S801	qnrD	aac(6)-lb-cr
18M 26(S) 32(S) 29(S) 31(S) 2(S)	32(S)	4(S)	0.06(S)	0.125(S)	0.06(S)	wt	wt	dnrD	aac(6)-lb
19M 24(S) 35(S) 30(S) 35(S) 4(S)   21M 25(S) 35(S) 33(S) 36(S) 4(S)   21M 25(S) 35(S) 33(S) 36(S) 4(S)	31(S)	2(S)	0.125(S)	0.06(S)	0.06(S)	wt	wt	ı	aac(6)-lb-cr
21M 25(S) 35(S) 33(S) 36(S) 4(5 22M 206(S) 27(S) 24(S) 26(S) 24(S)	35(S)	4(S)	0.125(S)	0.125(S)	0.06(S)	wt	wt	ı	aac(6)-lb-cr
	36(S)	4(S)	0.125(S)	0.125(S)	0.06(S)	wt	wt	dnrD	aac(6)-lb-cr
-)7 (c)0c (c)+c (c)/c (c)07 MIZZ	36(S)	2(S)	0.06(S)	0.06(S)	0.06(S)	wt	wt	ı	aac(6)-lb
23M 29(S) 38(S) 34(S) 36(S) 2(S	36(S)	2(S)	0.06(S)	0.06(S)	0.06(S)	wt	wt	ı	aac(6)-lb
24M 23(S) 32(S) 29(S) 30(S) 4(5	30(S)	4(S)	0.125(S)	0.125(S)	0.06(S)	wt	wt	dnrD	aac(6)-lb-cr
25M 12(R) 37(S) 34(S) 35(S) 32	35(S)	32(R)	0.125(S)	0.25(S)	0.125(S)	wt	wt	qnrD	aac(6)-lb-cr
32M 0 (R) 0 (R) 9 (R) 25	9 (R)	256 (R)	<u>&gt;</u> 32 (R)	<u>&gt;</u> 32 (R)	16 (R)	S463A, S464Y, E466D	S801	qnrD	aac(6)-lb-cr
33M 21(S) 34(S) 27(S) 31(S) 16	31(S)	16(S)	0.25(S)	0.5(S)	0.125(S)	S463A	wt	qnrD	aac(6)-lb-cr

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Ser463Ala, Ser464Tyr and novel Glu466Asp substitutions while, 2 isolates exhibited only Ser463Ala substitution. The Ser80lle substitution in QRDR of *parC* occurred only in 14% (3/22) of the isolates, where 86% (19/22) of the isolates were harboring wild-type *parC*. Three isolates which were resistant to all tested quinolones demonstrated *gyrB* and *parC* QRDR substitutions simultaneously (Table 3).

# Detection of qnrA, qnrB, qnrS, qnrC, qnrD and aac(6')-Ib-cr

**genes:** The *qnrD* gene appeared to be the most prevalent where 59% (13/22) of the total isolates produced amplimers for the *qnrD* but, no positive amplification for *qnrA*, *qnrB*,

*qnrS, qnrC* was detected in any of the isolates. Although the *aac(6')-lb* was amplified in all the isolates, only 50% (11/22) of them were confirmed as *cr* variant by sequencing (Table 3).

**Phylogenetic comparison of** *gyrB* **sequences:** Phylogenetic tree derived by analyzing and comparing the *gyrB* gene sequences obtained by present study and the published NCBI sequences is illustrated in Fig. 1. Neighbor-joining phylogenetic tree indicate two distinct clads comprising first; present study isolates together with a quinolone resistant *M. morganii* human clinical isolate and the second; environmental isolates of *M. morganii*.



Fig. 1: Neighbor-joining phylogenetic tree derived by analyzing *M. morganii gyrB* sequences obtained from the current study and downloaded from NCBI

Sequences referred to AB972375.1, DQ360899.1, KF732712.1 and HM122057.1 were obtained from NCBI public database and the rest of the sequences were acquired from the current study, 1, 2: Major clads

### DISCUSSION

The trade of pet turtles has generalized with increasing number of aquariums, pet shops, online shops and pet cafes. As the pet trade thrives, careless management of pet shops, as well as high rearing density, can be the cause of emerged potential zoonotic bacteria<sup>6</sup>. Pet turtles have been reported as a reservoir for several opportunistic pathogenic bacteria, such as Salmonella spp., *Citrobacter* spp. and Aeromonas spp.<sup>1,19</sup>. But, there have been very few reports on *M. morganii* isolated from pet turtles. Epidemiological studies have revealed that *M. morganii* is frequently isolated from human clinical cases of nosocomial bacterial infections with emerged resistance mechanisms against quinolones<sup>9</sup>.

In the present study, 22 isolates were identified as *M. morganii*. The majority of these pet turtle-associated *M. morganii* isolates demonstrated a lower frequency of resistance to quinolones. In MIC break points of this study, three isolates demonstrated resistance to nalidixic acid, ciprofloxacin, ofloxacin and levofloxacin while only one isolate showed resistance to only nalidixic acid. In contrast, 18 (82%) isolates were susceptible for all tested quinolones. Similar outcomes have been reported earlier, indicating that quinolone-susceptible isolates (75%) predominated in *M. morganii* isolated from patients<sup>22</sup>. Albornoz *et al.*<sup>23</sup> also reported *M. morganii* clinical isolates showing 42% resistance to quinolones.

A specific primer that can be used to amplify the gyrA of M. morganii has not been reported so far. The studies of Mazzariol et al.<sup>24</sup> and Yaiche et al.<sup>10</sup> could not amplify gyrA in M. morganii and it was suggested that this is due to the unknown genome characteristics of this bacterium. However, Ser463Ala, Ser464Tyr and novel Glu 466Asp substitutions in gyrB were detected in all 3 quinoloneresistant isolates while Ser463Ala substitution was detected in 2 guinolone-susceptible isolates. None of the other susceptible isolates had mutations. To date, none of animal associated *M. morganii* was described with *qyrB* mutations. This study described for the first time, a new *gyrB* mutation in M. morganii. In Gram-negative bacilli, gyrB mutations are rare and they have been described at amino acid positions 426, 431, 447, 463, 464 and 466<sup>18</sup>. The Ser463Ala substitution has been reported in a clinical isolate of *M. morganii* in Tunisia<sup>10</sup>.

The substitution of Ser80lle in *parC* was observed in only 3 quinolone-resistant isolates. All the quinolone-susceptible isolates did not show any substitution in *parC* which aligns with the results of previous study<sup>23</sup>. Double target substitutions of QRDR (*gyrB-parC*) were detected in 12%

(3/22) of the isolates. Substitutions of Ser463Ala, Ser464Tyr and novel Glu466Asp in the *gyrB* QRDR coupled with Ser80lle in the *parC* QRDR conferred high levels of quinolone resistance in *M. morganii* isolates. More or less similar outcome was reported, in which clinical strain of *M. morganii* harbored two *gyrB* substitutions (Ser463Ala, Ser464Tyr) and one *parC* substitution (Ser80lle)<sup>10</sup>. However, several studies reported only single substitution in *gyrB* coupled with a *parC* substitution<sup>22,23</sup>.

The *qnr* proteins obstruct the action of quinolones on bacterial DNA gyrase and topoisomerase IV. Generally, the *aac(6')-lb* gene causes the resistance to aminoglycosides, but *aac(6')-lb-cr* encodes a variant of the aminoglycoside acetyltransferase which can confer to reduced susceptibility of quinolones<sup>11</sup>. The PMQR determinants lead to low-level resistance compared to QRDR mutations although, their dissemination between bacteria and the simultaneous presence of two or more resistance determinants in the same microorganism has an additive effect of increasing quinolone MIC values<sup>25,26</sup>.

This study reports the high prevalence of *qnrD* gene in pet turtle-associated *M. morganii* strains. Especially, 59% (13/22) of *M. morganii* of current study harbored *qnrD* gene while, the *qnrD* gene has been reported frequently in *M. morganii* clinical isolates recovered from patients<sup>16,24</sup>. With regards to the horizontal transfer of PMQR determinants between human and turtle flora, that similarity points out a potential public health risk. The *aac(6')-Ib* amplimer could be obtained from all the *M. morganii* isolates and 50% (11/22) isolates were confirmed as *cr* variants. Interestingly, all the resistant isolates harbored both *aac(6')-Ib-cr* and *qnrD* genes. Similar results have been detected earlier where *aac(6')-Ib-cr* and *qnr* genes together conferring the quinolone resistance of clinical *M. morganii* isolates<sup>22,27</sup>.

Phylogenetic analysis of *gyrB* sequences clearly interprets the genetic similarity of *M. morganii* isolated from pet turtles with the quinolone-resistant human clinical isolate and how they diverge from environmental isolates. Meanwhile, quinolone-resistant isolates were clustered more closely with the quinolone-resistant human clinical isolate. Only four reference sequences could be used to construct the phylogenetic tree due to less availability of nucleotide sequences of *M. morganii gyrB* gene in GenBank.

As a whole, it is evident that pet turtle-associated *M. morganii* are harboring PMQR genes and mutations in the QRDR and their phenotypical expression is also pronounced. In emphasis, only three strains were resistant to all tested quinolones while harboring *gyrB* and *parC* mutations in

QRDR and *qnrD* and *aac(6')-lb-cr* PMQR genes concurrently. It suggests that the gene expression mechanisms might be involved positively in modulating the final MIC<sup>28</sup>.

In pet shops, rearing aquatic animals including ornamental fish and turtles with high density in closed systems can pave the way for the emergence of antibiotic resistance due to horizontal transfer of genetic elements. This can be a possible reason for the high prevalence of PMQR genes in both quinolone-resistant and susceptible M. morganii isolates. Previous studies have demonstrated a high prevalence of PMQR genes in aquatic environments<sup>19,29</sup>. Although the exposure of antimicrobials causes the emergence of antimicrobial resistance, the natural evolutionary response has correlated with complex and interlinking drivers which have not revealed completely. On the other hand, acquisition of antimicrobial resistance mechanisms does not necessarily compromise microbial fitness. Worldwide clonal spread and long-term persistence of resistant bacteria are also seen in the absence of direct antibiotic selection pressure<sup>30,31</sup>.

# CONCLUSION

This is the first study describing quinolone resistance determinants in *M. morganii* strains isolated from pet turtles with a high prevalence of *qnrD* gene. A novel mutation (Glu 466Asp) in the QRDR of the *gyrB* gene was detected. Profoundly, pet turtle may represent a potential source of quinolone-resistant *M. morganii* due to the high prevalence PMQR genes [*qnrD* and *aac(6')-lb-cr*] and mutations in the QRDR of *gyrB* and *parC*. These findings suggest that studies associated with quinolone resistance, particularly, the genetics-based resistance of *M. morganii* worth considering significant. To gain more perceptivity into the molecular characterization of quinolone-resistant *M. morganii* isolates, other possible mechanisms of resistance should also be investigated deeply.

## SIGNIFICANT STATEMENT

The present study evaluated the genetic context related to quinolone resistance of *M. morganii* isolated from several species of pet turtles reared under laboratory conditions. The outcomes pointed out the pet turtles posing a potential public health risk due to high prevalence of plasmid mediated *qnrD* and target gene alterations in chromosomal QRDR.

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