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Research Article
Characterization of Quinolone Resistance of *Pseudomonas aeruginosa* Isolated from Pet Chinese Stripe-necked Turtles (*Ocadia sinensis*)

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Abstract

Background and Objective: Infections of antibiotic-resistant bacteria in both human and animals own a great significance. The current study aimed to determine the quinolone susceptibility and the genetic characteristics of quinolone resistance of 20 strains of *Pseudomonas aeruginosa* isolated from pet Chinese stripe-necked turtles (*Ocadia sinensis*). **Methodology:** Susceptibility of four antimicrobials including nalidixic acid, ciprofloxacin, ofloxacin and levofloxacin was examined. The PCR was carried out to amplify Quinolone Resistance Determining Region (QRDR) and to screen Plasmid Mediated Quinolone Resistance (PMQR) genes. **Results:** All tested isolates were resistant to nalidixic acid (100%), but none of the isolates show complete resistance to other tested antimicrobials. Two isolates showed intermediate resistance to ciprofloxacin (5%) and ofloxacin (5%), one each in Minimum Inhibitory Concentration (MIC) test. The *qnrB* gene was identified in one isolate (5%) and *qnrS* in three isolates (15%). The PCR assay could amplify *aac(6')-Ib* gene from 8 isolates (40%) and none of them harbored *aac(6')-Ib-cr* variant. Sequences obtained by amplifying *gyrA* and *parC* regions did not show any point mutation in QRDR. Neighbor-joining phylogenetic tree for *gyrA* indicated three distinct clads comprising first, current study isolates, second, clinical isolates of human and dogs and third, isolates from soil and water. **Conclusion:** All results suggest that studied strains of *P. aeruginosa* are less resistant to quinolones and are genetically more conserved with regards to *gyrA* gene region.

Key words: *Pseudomonas aeruginosa*, quinolone resistance, pet Chinese stripe-necked turtle, *qnr* genes, QRDR

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

INTRODUCTION

Ubiquitous environmental bacterium, *Pseudomonas aeruginosa* is a Gram-negative, aerobic bacterium which mostly thrives in moist or wet conditions. As a human opportunistic pathogen, *P. aeruginosa* causes infections ranging from minor gastrointestinal infections resulting in diarrhea, fever and headache to severe infections including meningitis, bacterial keratitis, endophthalmitis and necrotizing enterocolitis, especially in patients with impaired immune systems[1]. Despite *Pseudomonas* spp. is considered part of the indigenous flora of chelonian oral cavity and intestinal tract, they can cause opportunistic infections leading to ulcerative stomatitis, dermatitis, septicemia and pneumonia[2].

Aminoglycosides and fluoroquinolones are commonly used for the systemic treatment of *P. aeruginosa* infections. However, resistance to these drugs is frequently detected in clinical practice[3]. The *P. aeruginosa* has been identified to possess an intrinsic antimicrobial resistance due to low outer membrane permeability, chromosomally encoded cephalosporinase and an extensive efflux pump system[4]. Besides, *P. aeruginosa* holds a prominent place in development of acquired resistance through horizontal transfer of genetic elements (integrons, transposons and plasmids) which contain resistance genes as well as by chromosomal mutations in DNA gyrase and topoisomerase IV[5,6].

Plasmid mediated quinolone resistance develops mainly through the production of *qnr* proteins[7]. First *qnr* determinant was introduced as *qnrA* and the subsequent were known as *qnrB*, *qnrC*, *qnrS* and *qnrD*[8]. Furthermore, an enzyme called *aac(6')-Ib* causes aminoglycoside resistance by acetylation and a variant of this enzyme, *aac(6')-Ib-cr* was found to be another cause of plasmid-mediated quinolone resistance[9]. Mutations in DNA gyrase and topoisomerase IV arise most commonly in *gyrA* and *parC* subunits, respectively[10]. Because of the consistent occurrence of the mutations in *gyrA* region from amino acid position 67-106, it is commonly referred to as Quinolone Resistance Determining Region (QRDR)[11].

Chelonians, comprised of turtles, terrapins and tortoises are kept in captivity for commercial purposes, particularly as pets. So far, turtle keeping has been continuously growing but it has not been matched with an appropriate increase in medical knowledge[12]. Pet turtles have been known to harbor a variety of zoonotic bacteria, especially in their digestive tract[13]. Among these, *Salmonella* spp., in particular have been well-studied as a source of salmonellosis in humans[14,15]. In the meantime, *P. aeruginosa* has been isolated from sea turtles and desert tortoises[16,17]. A MLST analysis has been conducted for the *P. aeruginosa* isolated from pet CSN turtles[18]. Moreover, *P. aeruginosa* isolated from pet turtles and turtle eggs exhibited a high-level aminoglycoside resistance[19].

Chinese Stripe-Necked (CSN) turtle (*Ocadia sinensis*) is a medium-sized geoemydid turtle (up to 24 cm carapace length) with numerous dark-bordered, narrow yellow stripes on the head and neck. The CSN turtles are recognized as native inhabitants of Taiwan, Southern China and Northern Vietnam[20]. But, they are now being distributed worldwide and also imported to South Korea as a pet. The CSN turtle shows an omnivorous dietary habit which is surprisingly tolerant of harsh and even polluted aquatic systems[20]. Hence, ease of keeping could be the reason CSN to become more popular as a pet.

Current study sought to characterize the *P. aeruginosa* isolated from pet CSN turtles through the detection of quinolone susceptibility and screening the plasmid-mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrS* and *aac(6')-Ib-cr*) and chromosomal QRDR mutations in *gyrA* and *parC* for a better understanding of an opportunistic pathogen in pet turtles and potential zoonotic pathogen in human.

MATERIALS AND METHODS

Purchase of pet turtles: A total of 40 Chinese stripe-necked turtles were purchased from several pet shops and online markets in Korea. The turtles were under 4 weeks of age at purchase and studied immediately. All turtles were clinically inspected upon purchase and showed no signs of disease. Turtles were raised under laboratory conditions following the general husbandry method[21].

Isolation of *P. aeruginosa*: Cloacal swabs from turtles were enriched by submerging them in Tryptic Soy Broth (TSB) and incubating at 37°C for 24 h. Enriched samples were streaked onto the selective culture medium cetrimide agar (CN) and incubated at 37°C for 24 h. Suspicious colonies were subcultured onto CN agar and incubated once more at 42°C for 24 h. The isolates that showed the positive growth were presumptively identified as *P. aeruginosa*.

Bacterial identification using 16S rRNA gene amplification and sequencing: Genomic DNA was extracted from presumptively identified *P. aeruginosa* 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.

Quinolone susceptibility testing: Twenty out of 31 identified isolates were randomly selected for the study. Susceptibility pattern of the *P. aeruginosa* isolates was investigated for nalidixic acid, ciprofloxacin, ofloxacin and levofloxacin by disk diffusion test on Mueller Hinton agar (MBcell Ltd., Seoul, Korea). Minimum Inhibitory Concentrations (MIC) of nalidixic acid, ciprofloxacin and ofloxacin were detected by broth microdilution method. All susceptibility testing were conducted according to the recommendations of Performance Standards for Antimicrobial Susceptibility Testing, Clinical and Laboratory Standards Institute[22].

Detection of quinolone resistance genes: The presence of *qnrA*, *qnrB* and *qnrS* genes were detected using primers and PCR conditions listed in Table 1. Each PCR mixture was 20 µL total volume comprising of 10 µL of Quick Taq® HS DyeMix (Toyobo, Japan) 1 µL of 10 pmol μL^{-1} each primer and 1 µL of the template. The PCR products were checked in 1.5% agarose gel.

Detection of *aac(6')-Ib-cr* variant: The PCR was performed to amplify the *aac(6')-Ib* gene region by using the same protocol used for *qnr* genes (Table 1). Amplicons were purified using Expin™ PCR SV kit (GeneAll®, Korea) and were sent to Cosmogenetech Co., Ltd., Daejeon, Korea for sequencing. Acquired sequences were tested for the similarity in BLAST option in NCBI database in order to check the presence of *aac(6')-Ib-cr* gene variant.

Amplification of Quinolone Resistance Determining Region (QRDR) and detection of mutations: The PCR for *gyrA* and *parC* regions were performed using primers and conditions described in Table 1. The PCR was conducted in 50 µL volumes consisting of 20 µL of Quick Taq® HS DyeMix (Toyobo, Japan), 2 µL of 10 pmol μL^{-1} each primer and 2 µL of the template. Amplicons were purified using Expin™ PCR SV kit (GeneAll®, Korea) and sent to Cosmogenetech Co., Ltd., Daejeon, Korea for sequencing. Acquired sequences were subjected to detection of mutations by comparison with published NCBI reference sequences (Accession No. L29417.1 and AB003428.1 for *gyrA* and *parC*, respectively) as described by Rubin *et al.*[23]. Analyzing and comparison of QRDR sequences were performed using Mutation Surveyor V5.0.1 (Softgenetics LLC, USA) software.

Table 1: Primers used to amplify QRD, *qnr* genes and conditions of each reaction

				PCR conditions					
Target	Primer	Nucleotide Sequence (5'-3')	Size (bp)	Pre-denaturation	Denaturation	Annealing	Elongation	Final elongation	Source of primer sequence
gyrA	*gyrA*-F	AGTCCTATCTCGACTACGCGAT	330	95°C, 3 min[a]	95°C, 1 min, 35x	58°C, 45 sec, 35x	72°C, 1 min, 35x	72°C, 7 min	Akasaka *et al.*[24]
	gyrA-R	AGTCGACGGTTTCCTTTCCAG							
parC	*parC*-F	CTGGATGCCGATTCCAAGCAC	186	95°C, 3 min[a]	95°C, 1 min, 35x	58°C, 45 sec, 35x	72°C, 1 min, 35x	72°C, 7 min	Mouneimne *et al.*[25]
	parC-R	GAAGGACTTGGGATCGTCCGG							
qnrA	*qnrA*-F	ATTTCTCACGCCAGGATTTG	516	94°C, 5 min[b]	94°C, 45 sec, 32x	53°C, 45 sec, 32x	72°C, 1 min, 32x	72°C, 7 min	Robicsek *et al.*[9]
	qnrA-R	GATCGGCAAAGGTTAGGTCA							
qnrB	*qnrB*-F	GATCGTGAAAGCCAGAAAGG	463	94°C, 5 min[b]	94°C, 45 sec, 32x	54°C, 45 sec, 32x	72°C, 1 min, 32x	72°C, 7 min	Robicsek *et al.*[9]
	qnrB-R	ACGATGCCTGGTAGTTGTCC							
qnrS	*qnrSm*-F	GCAAGTTCATTGAACAGGGT	428	95°C, 5 min[a]	95°C, 1 min, 35x	58°C, 1 min, 35x	72°C, 1 min, 35x	72°C, 7 min	Cattoir *et al.*[26]
	qnrSm-R	TCTAAACCGTCGAGTTCGGCG							
aac(6')-Ib	*aac(6')-Ib-F*	TTGCGATGCTCTATGAGTGGCTA	482	94°C, 5 min[a]	94°C, 45 sec, 35x	55°C, 45 sec, 35x	72°C, 45 sec, 35x	72°C, 10 min	Park *et al.*[27]
	aac(6')-Ib-R	CTCGAATGCCTGGCGTGTTT							

[a]PCR conditions were modified for this study, [b]PCR conditions were adapted from the reference source

Table 2: Details of the *gyrA* gene sequences of *P. aeruginosa* downloaded from NCBI database for the phylogenetic analysis

NCBI accession number	Origin	Country of record
EU753434.1	Quinolone-resistant human clinical isolate	China
EU753435.1	Quinolone-resistant human clinical isolate	China
KU522119.1	Human clinical isolate	Iran
KU380335.1	Human clinical isolate	Iran
KP281423.1	Antibiotic resistant clinical isolate from dogs with otitis externa and pyoderma	Brazil
KP281423.1	Antibiotic resistant clinical isolate from dogs with otitis externa and pyoderma	Brazil
JQ844605.1	Ciprofloxacin resistant isolates of soil samples	Southern India
JQ844604.1	Ciprofloxacin resistant isolates of soil samples	Southern India
KJ920445.1	Quinolone resistant isolate from water	Brazil
KJ920446.1	Quinolone resistant isolate from water	Brazil

Table 3: Antimicrobial susceptibility pattern and genetic characterization of quinolone resistance of *Pseudomonas aeruginosa* isolated from pet Chinese stripe-necked turtles

	Disk diffusion zone diameters (mm)				MIC (μg mL^{-1})			Genetic characterization		
Isolates	NDX30	CIP5	OFX5	LEV5	NDX	CIP	OFX	*qnrB*	*qnrS*	*aac(6')-Ib*
N01	0 (R)	30 (S)	21 (S)	26 (S)	128 (R)	0.25 (S)	2 (S)	-	-	-
N03	0 (R)	24 (S)	20 (S)	22 (S)	256 (R)	0.5 (S)	4 (I)	-	-	-
N04	8 (R)	33 (S)	24 (S)	24 (S)	256 (R)	1 (S)	2 (S)	-	-	-
N05	8 (R)	27 (S)	22 (S)	26 (S)	256 (R)	0.25 (S)	2 (S)	-	-	-
N07	8 (R)	32 (S)	27 (S)	22 (S)	512 (R)	0.5 (S)	2 (S)	-	-	-
N12	0 (R)	40 (S)	30 (S)	25 (S)	256 (R)	0.25 (S)	2 (S)	-	-	*aac(6')-Ib*
N15	0 (R)	36 (S)	28 (S)	24 (S)	128 (R)	0.25 (S)	1 (S)	-	-	*aac(6')-Ib*
N20	0 (R)	39 (S)	28 (S)	23 (S)	128 (R)	0.25 (S)	2 (S)	-	-	-
N22	0 (R)	38 (S)	30 (S)	23 (S)	128 (R)	0.25 (S)	2 (S)	-	-	-
N24	0 (R)	35 (S)	26 (S)	22 (S)	256 (R)	1 (S)	2 (S)	-	-	*aac(6')-Ib*
N25	0 (R)	38 (S)	29 (S)	23 (S)	256 (R)	0.25 (S)	2 (S)	-	-	*aac(6')-Ib*
N26	0 (R)	37 (S)	25 (S)	22 (S)	128 (R)	0.5 (S)	2 (S)	-	*qnrS*	-
N28	0 (R)	38 (S)	28 (S)	24 (S)	128 (R)	2 (I)	2 (S)	*qnrB*	-	-
N29	0 (R)	38 (S)	27 (S)	28 (S)	128 (R)	0.25 (S)	2 (S)	-	*qnrS*	-
N30	0 (R)	35 (S)	27 (S)	21 (S)	128 (R)	0.125 (S)	1 (S)	-	-	-
N31	0 (R)	37 (S)	30 (S)	21 (S)	128 (R)	0.25 (S)	1 (S)	-	-	*aac(6')-Ib*
N32	0 (R)	27 (S)	19 (S)	22 (S)	256 (R)	0.5 (S)	2 (S)	-	-	*aac(6')-Ib*
N33	0 (R)	36 (S)	30 (S)	26 (S)	128 (R)	0.25 (S)	2 (S)	-	-	*aac(6')-Ib*
N34	0 (R)	34 (S)	27 (S)	21 (S)	256 (R)	0.25 (S)	2 (S)	-	-	*aac(6')-Ib*
N35	0 (R)	37 (S)	27 (S)	23 (S)	256 (R)	0.5 (S)	2 (S)	-	*qnrS*	-

NDX30: Nalidixic acid (30 μg), CIP5: Ciprofloxacin (5 μg), OFX5: Ofloxacin (5 μg), LEV5: Levofloxacin (5 μg), S: Susceptible, I: Intermediate, R: Resistant were designated using breakpoints described by the Clinical Laboratory Standards Institute[22]

Phylogenetic comparison of *gyrA* sequences with published sequences of different origin: The sequences derived for *gyrA* gene region were analyzed and a neighbor-joining phylogenetic tree was obtained with 1000 bootstrap replications. For the analysis, 10 *gyrA* sequences of *P. aeruginosa* of different origin (i.e., clinical and environmental) were obtained from published sequences of the GenBank (Table 2 for the accession numbers and details) and MEGA6 sequence analyzing software was used for aligning and construction of the phylogenetic tree.

Submission of DNA sequences to NCBI: A total of nine representative sequences, including five *gyrA* and four *aac(6')-Ib*, acquired from the current study were submitted to GenBank.

RESULTS

Isolation and identification of *P. aeruginosa*: Thirty-one of forty isolates showed positive growth on CN agar at 42°C. A subsequent BLAST search after 16S rRNA sequencing indicated a 99-100% match to *P. aeruginosa* sequences available in GenBank which confirmed their identity as *P. aeruginosa*.

Quinolone susceptibility testing: The results of the quinolone susceptibility testing are shown in Table 3. Consistent with the results of both disc diffusion test and MIC test, all 20 (100%) isolates of *P. aeruginosa* showed resistance to nalidixic acid. In disc diffusion test, no isolate showed intermediate resistance or resistance to other three quinolones,

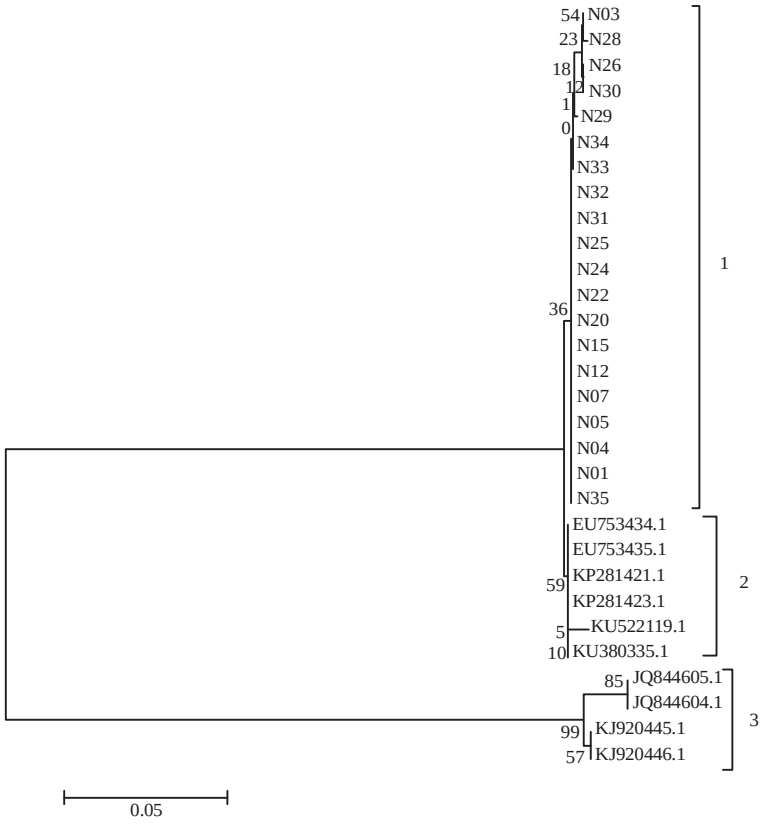

Fig. 1: Neighbor-joining phylogenetic tree derived by analyzing *P. aeruginosa gyrA* sequences obtained from the current study and downloaded from NCBI, sequences referred to as EU753434.1, EU753435.1, KP281423.1, KP281423.1, KU522119.1, KU380335.1, JQ844605.1, JQ844604.1, KJ920445.1 and KJ920446.1 were obtained from NCBI public database and the rest of the sequences were acquired from the current study, 1, 2, 3: Major clads

Table 4: Accession numbers of the *gyrA* and *parC* sequences which were submitted to NCBI

GenBank accession number	Genotype	Corresponding isolate
KY354010	*gyrA*	N01
KY354011	*gyrA*	N22
KY354012	*gyrA*	N24
KY354013	*gyrA*	N25
KY354014	*gyrA*	N34
KY319091	*aac(6')-Ib*	N24
KY319092	*aac(6')-Ib*	N25
KY319093	*aac(6')-Ib*	N31
KY319094	*aac(6')-Ib*	N33

ciprofloxacin, ofloxacin and levofloxacin. In MIC test, two isolates, N03 and N28 showed intermediate resistance to ciprofloxacin (5%) and ofloxacin (5%) respectively (Table 3).

Detection of quinolone resistance genes (*qnrA, qnrB, qnrS* and *aac(6')-Ib-cr*) and QRDR mutations: Table 3 shows that, none of the isolates possessed *qnrA* gene and only one isolate referred to as N28 harbored *qnrB* gene (5%). Three isolates showed *qnrS* gene (15%), while *aac(6')-Ib* could be amplified from 8 isolates (40%) (Table 3). However, sequencing results could not identify any *aac(6')-Ib-cr* variant in BLAST comparison. Neither *gyrA* nor *parC* sequences exhibited mutations in QRDR, within amino acid positions 67-106.

Phylogenetic comparison of *gyrA* sequences: Phylogenetic tree derived by analyzing and comparing the *gyrA* gene sequences obtained by current study and the published NCBI sequences is illustrated in Fig. 1. Neighbor-joining phylogenetic tree indicated three distinct clads comprising, clad 1 current study isolates, clad 2 clinical isolates of human and dogs and clad 3 isolates from soil and water.

Submission of DNA sequences to NCBI: Accession numbers of the *gyrA* and *parC* sequences which have been deposited in the GenBank database were listed in Table 4.

DISCUSSION

It is much reasonable to deem that *P. aeruginosa* is an opportunistic pathogen which is able to cause serious infections in animals and human[1,2]. In the meantime, it's intrinsic and acquired resistance to antimicrobials has also been well studied[4,6–8]. Due to higher significance and implications counting current health concerns, studies associated with quinolone resistance, particularly, genetics-based resistance of *P. aeruginosa* are more pronounced[23,28-30].

Yonezawa *et al*.[31] have studied the *gyrA* mutations of quinolone-resistant *P. aeruginosa* clinical isolates in the early era. Descending from such studies numerous similar studies have been conducted[29,32,33]. Parallel clinical studies related to animals were directed to canine infections of dogs and isolates from poultry[23, 30,34]. Meanwhile, a study focused on pet turtles encountered a range of antimicrobial-resistant bacteria including *P. aeruginosa* which showed a high-level aminoglycoside resistance, particularly to gentamicin[19]. However, to our knowledge, quinolone resistance studies regarding *P. aeruginosa* from pet turtles have not been conducted so far.

Being consistent with above, the current study was conducted to characterize the *P. aeruginosa* isolated from pet CSN turtles. The CSN turtle is a common species of pet turtles being kept by South Korean people, hence there is a possibility of getting opportunistic infections into humans who handle the turtles and on the other hand, might be a potential pathogen to the turtle itself when they are immunologically compromised.

In accordance with the disk diffusion and MIC outcomes, 100% resistance to nalidixic acid was observed. More or less similar outcome was observed with 96% (102/106 isolates) resistance to nalidixic acid from canine infected dog isolates[23]. In contrast, Al-Marjani[35] reported *P. aeruginosa* clinical isolates showing 42.1 and 39.4% resistance to levofloxacin and nalidixic acid, respectively where environmental isolates showed the highest resistance rate (27.5%) to nalidixic acid. But, isolates of this study did not show any resistance to tested fluoroquinolones, ciprofloxacin, ofloxacin and levofloxacin. In line with this, a previous study reported *P. aeruginosa* isolated from a river which showed a 100% susceptibility to ciprofloxacin and levofloxacin[36]. Besides, multidrug-resistant clinical isolates from pediatric patients could show 94.8% (55/58) resistance to both ciprofloxacin and levofloxacin[37]. However, MIC results indicated two isolates showing intermediate resistance, isolate N03 to ofloxacin and isolate N28 to ciprofloxacin but only N28 could show *qnrB* gene. In the meantime, Michalska *et al*.[28] have detected *qnrB* as the most prevalent in *P. aeruginosa* but Cayci *et al*.[38] could not detect any of the *qnr* genes even in resistant strains. However, another study reported the presence of both *qnrS* and *qnrA* simultaneously in clinical samples (21% *qnrS* and 13.1% *qnrA*), while 2.5% (1/40) *qnrS* could be observed in sewage and tap water isolates[35]. In this study, three isolates which harbored *qnrS* were susceptible to all tested quinolones, except nalidixic acid. Even though it is controversial, a similar kind of observation was reported, in which *Aeromonas* spp., isolated from freshwater fishes harbored *qnr* genes and QRDR mutations, despite they are susceptible to quinolones[39]. Therefore, the necessity of investigating the organismal factors and mechanisms which could alter the gene expression owns significance. In the case of *aac(6')-Ib* gene, none of the isolates could show the *aac(6')-Ib-cr* variant, denoting that quinolone resistance is not mediated through *aac(6')-Ib-cr* gene. Therefore, it could be a sign of aminoglycoside resistance as Diaz *et al*.[19] and Michalska *et al*.[28] reported but not plausible without further experiments.

A few isolates showed *qnr* genes but no QRDR mutations in both *gyrA* and *parC* regions were detected in any of the isolates. Although, every isolate showed resistance to nalidixic acid, a substantial resistance to fluoroquinolones was not detected. Because, plasmid-mediated *qnr* genes are not strong enough to show a phenotypic resistance unless it is facilitated by QRDR chromosomal mutations[7]. In Gram-negative bacteria, DNA gyrase seems to be the primary cellular target of quinolones, particularly fluoroquinolones. With regards to *P. aeruginosa gyrA*, the most common mutations that lead to drug resistance occur at Thr83 and Asp87 of the subunit[11]. In a human clinical study, 50% (19/38) of the ciprofloxacin-resistant strains harbored *gyrA* Thr83Ile mutation and *parC* Ser80Leu mutation[40]. Some other animal and human clinical studies also reported a range of QRDR mutations, majorly in *gyrA* and *parC* in which the isolates were showing a higher resistance to quinolones[23,29]. Although, some non-specific silent mutations of the amplified region were noticed, current study could not identify any of above mentioned specific point mutations within the amino acid positions 67-106. Therefore, it can be stated that QRDRs of every isolate in this study isolates is more conserved compared to highly resistant isolates.

According to the personal communication with pet shops, the studied group of turtles was very young and aged less than 4 weeks. All the individuals were purchased soon

after hatched did not show any sign of disease and were confirmed not exposed to any antibiotics. It could be the reason *P. aeruginosa* isolates to become more susceptible to quinolone antibiotics. Nevertheless, a few isolates show *qnr* genes in their genome. Since those mediators are transferable from other organisms, two possible causes can be addressed. One is those plasmids might have horizontally transferred from other bacterial species while the newly hatched turtles were kept together with other turtles in the same cage till purchase. And the second is those strains could get plasmids from other bacterial species which thrive in the turtle's own body.

Phylogenetic analysis of *gyrA* sequences produced a neighbor-joining tree showing 3 major clads referred to as 1, 2 and 3. Interestingly, clad 1 comprised of isolates of the current study, clad 2 contained all clinical isolated both from human and pet dogs. The isolates from water and soil were separated as last clad, 3. This outcome clearly interprets the genetic similarity of *P. aeruginosa* isolated from CSN turtles and how they diverge from clinical and environmental isolates. As the phylogenetic tree portrays, the environmental isolates represent the basal clad and all other isolates separated arising from it. It might be caused by the source of bacteria separating animal isolates from environmental. A comparative genomic study also reported separated clusters in dendrogram revealing the genetic difference among human, bovine and environmental isolates of *P. aeruginosa*[41]. Although clinical and current study isolates are more or less closely related than with environmental isolates, still they keep in two distinct clads revealing that isolates from these young CSN turtles are genetically more unique and conserved than quinolone-resistant clinical isolates. Bootstrap node support for the separation of clinical isolates and current study isolates were 59 and 36% respectively. Furthermore, it is important to observe the 99% bootstrap separation of isolates from soil samples and water in clad 3, revealing the genetic diversity of *P. aeruginosa* even between water and land.

CONCLUSION

It is plausible that the *P. aeruginosa* isolated from CSN turtles are not resistant to tested fluoroquinolones and their QRDRs were genetically more conserved than that of quinolone-resistant strains. So it can be a plus point for the people who keep pet CSN turtles but further studies regarding more turtle species and more antimicrobials are highly recommended for a better understanding.

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