Veterinary Integrative Sciences 2022; 20(3): 761 - 773 DOI; 10.12982/VIS.2022.059

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Pseudomonas aeruginosa from pet Chinese stripe-necked turtles (*Ocadia sinensis*) demonstrating antimicrobial and heavy metal resistance

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Abstract

Leading nosocomial pathogen *Pseudomonas aeruginosa* has increasingly been reported to be an opportunistic pathogen. In this study, a total of twenty *P. aeruginosa* isolates were isolated from 40 pet Chinese stripe-necked turtles and examined for their antimicrobial and heavy metal resistance properties. All isolates were multidrug resistance by scoring multiple antimicrobial resistance indices ≥0.2. In the disc distribution test, 100% resistance to ampicillin and oxacillin were detected. In addition to that, 14 (70%) isolate demonstrated amoxicillin resistance. Imipenem, fosfomycin, gentamycin, tobramycin and piperacillin resistance were detected in 40%, 15%, 20%, 10% and 5% of the isolates, respectively. The ESBLs gene that predominated in this study was *blaSHV* (55%), followed by *blaTEM* (50%), *blaCTX* (10%) and *blaOXA* (5%). The most frequent aminoglycoside resistance gene in this study was *aac*(6′)-*Ib* (40%). Class1 integron integrase gene *intI1* and class 1 integron gene cassette gene *aadA1* were detected in 45% and 35% of the isolates, respectively. All *P. aeruginosa* isolates demonstrated Cu and Cd resistance. *CzcA* and *CopA* genes were detected in 65% and 30% of the isolates, respectively. These findings reveal the presence of pet turtle-born *P. aeruginosa* can be a potential risk to public health and cannot be excluded as a non-nosocomial source of infections.

Keywords: Antimicrobials, Heavy metals, *Ocadia sinensis*, Pet turtles, *Pseudomonas aeruginosa*

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Funding; This research was supported by Vingroup Innovation Foundation (VINIF) in project code VINIF.2020.DA05.

Article history; *received manuscript: 23 June 2022, revised manuscript: 1 August 2022, accepted manuscript: 10 October2022, published online:25 October 2022*

Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, monoflagellated bacterium. It is an ubiquitous bacterium and has been identified from various niches including the natural environment and health-care settings (Ramsay et al., 2019). As an opportunistic pathogen, *P. aeruginosa* can cause a range of infections in humans who are immunocompromised or have predisposing conditions. Pneumonia, bacteremia, urosepsis, wound infection and secondary infection of burns are among the *P. aeruginosa* infections (Kerr and Snelling, 2009).

P. aeruginosa is among the six ESKAPE pathogens that cause nosocomial infections frequently in the US and are a threat all over the world (Lupo et al., 2018). Transmission occurs by direct contact with contaminated reservoirs, ingestion of contaminated materials and also, there are some pieces of evidence about air-bone transmissions (Clifton and Peckham, 2010). Comparatively large substantially conserved genome in *P. aeruginosa* is the foundation of the various adaptive mechanisms. This triggers the rapid adaptation and persistence in any environment (Schmidt et al., 1996).

Reptiles' popularity as mainstream pets has grown rapidly. Among them, tortoises and turtles are the most common reptiles living in the domestic environment. However, they may harbor and excrete a large variety of zoonotic pathogens such as *Salmonella* spp., *Mycobacterium* spp., *Aeromonas* spp., *Citrobacter* spp. and *Pseudomonas* spp. (Ebani, 2017). *P. aeruginosa* has been isolated from pet turtles, desert tortoises, and sea turtles. Moreover, pet turtle-borne *P. aeruginosa* has demonstrated its virulence and antimicrobial resistance properties. Particularly, quinolone and aminoglycoside resistance (Silva et al., 2017).

Chinese stripe necked (CSN) turtles have gained popularity among the pet turtle lovers because of their attractive characters as a pet. CSN turtles are relatively small in size (carapace length below 24cm) and have attractive narrow yellow stripes on the neck and head regions. Chinese stripe-necked turtles (*Ocadia sinensis*) are recorded as inhabitants of lakes, rivers, and canals (Di Blasio, 2021). Omnivorous dietary habit and high tolerance even in deteriorated environments, as well as their calm and less aggressive behavior, gives fewer rearing difficulties to pet lovers. This species is native to East-Asian countries. However, they are being introduced to other countries as a pet animal including South Korea (Grzime, 2003). These healthy turtles can transmit pathogenic bacteria through their feces because they may shed bacteria with their feces (Di Ianni et al., 2015). It was reported in a previous study done using healthy pet turtles which showed bacterial contamination in turtle fecal materials (Hossain et al., 2017). Pet-turtle-borne pathogenic bacteria can be transmitted to the human from turtles and turtle tanks to humans. (Diaz et al., 2006). In addition, bacteria can be transmitted through contaminated environments such as poorly maintained pet shops when hygienic factors are neglected (Stam et al., 2003).

Molecular characteristics including virulence, antimicrobial resistance and heavy metal resistance properties play a significant role in microbial pathogenicity (Jiang et al., 2020; Qin, 2022). Plasmids, integrons, and transposons, which are mobile genetic components, help bacteria spread their genetic resistance determinants more widely (Boerlin and Reid-Smith, 2008).

From those elements, mobile integrons recombine gene cassette arrays facilitate bacteria to distribute antimicrobial resistance genes, and develop multidrug resistance properties (Deng et al. 2015). The class 1 integrons are one of the integron classes consisting of class 1, class 2 and class 3 integrons.

Bacteria have developed several types of resistance mechanisms to overcome heavy metal stress for their survival. Among those mechanisms, formation and sequestration of heavy metals in complexes, metal reduction to a less toxic species, and metal efflux mechanisms were mainly reported (Teitzel and Parsek, 2003). Heavy metal-resistant bacteria can cause co-resistance cross-resistance to antimicrobials or vice versa. Chromosomal elements and plasmids can facilitate heavy-metal resistance abilities of bacterial pathogens (Mariana Ramos et al., 2018). Therefore it is important to study about molecular characteristics of *P. aeruginosa* to understand its potential risk.

The number of studies that have been conducted in Korea to determine the virulence and antimicrobial resistance patterns of bacterial species isolated from pet turtles are limited. No study has been conducted to characterize antimicrobial resistance properties along with the gene cassettes and heavy metal resistance properties in *P. aeruginosa* isolated from pet turtles in Korea. Therefore, we designed this study to examine antimicrobial resistance determinants, mobile genetic elements, and heavy metal resistance in *P. aeruginosa* isolated from pet CSN turtles in order to reveal the ability to disseminate antimicrobial resistance in the domestic environment.

MATERIALS AND METHODS

Purchasing and raring turtles

Forty captive Chinese stripe-necked turtles under 4 weeks of age were purchased from different pet shops (Seoul) and online markets in South Korea randomly (Wendt and Heo, 2016). Turtles were inspected upon purchase to select healthy turtles. Turtles without any clinical signs of disease and treatment history were considered as healthy. Turtles were managed in the cages containing slope made of soils and pebbles and sterilized water. Water quality was maintained using a canister filter to maintain water quality. General husbandry methods were followed to raise turtles in the laboratory (Bluvias, 2010). Turtles were numbered N01 to N40 and they were transported separate containers and raised in separate cages to prevent cross contamination. However, raring conditions were same for all turtles.

Isolation and identification of *P. aeruginosa*

Samples were taken from the cloaca by using cotton swabs. Then the cloacal swabs were submerged in Tryptic Soy Broth (TSB) and incubated at 37ºC for 24 hours for enriching. Few drops from the enriched samples were streaked onto the cetrimide agar (CN) and incubated for 24 hours at 37ºC. Doubtful colonies were subcultured and incubated once more for 24 hours at 42ºC. Colonies that could grow at 42ºC were presumptively identified as *P. aeruginosa*. Genomic DNAs of the presumptively identified isolates were extracted by Chelex 100 extraction method and PCR for 16S rRNA was performed using universal primers 12F and 1492R. PCR products were sent to

Cosmogenetech Ltd. (Daejon, Korea) for gene sequencing. BLAST analysis tools in the NCBI database were employed to confirm species status (Wendt and Heo, 2016).

Antimicrobial susceptibility test and heavy metal tolerance assays

The disc diffusion method published by the Clinical and Laboratory Standards Institute (CLSI, 2014) was employed for examining susceptibility against 20 antimicrobial agents. The multiple antimicrobial resistance (MAR) indices were calculated according to the study published by Krumperman (1983). Twenty antimicrobials were selected as follows: Aminoglycosides; streptomycin (STR,10µg), kanamycin (KAN,30µg), gentamycin (GEN,10µg), tobramycin (TOB,10µg). Carbapenems; meropenem (MER,10µg), imipenem (IMI,10µg). Cephalosporins; cefotaxime (CEFO,30µg), cephalothin (KF,30µg), ceftriaxone (CRO,30µg), ceftazidime (CEFT,30µg), cefepime (CEP,30µg). Folate pathway inhibitors; trimethoprim-sulfamethoxazole (SXT,25µg). Penicillins; piperacillin (PRL,100µg), ampicillin (AMP,10µg), oxacillin (OXO,5 µg), ticarcillin (TIC,75µg), amoxicillin (AMO,30 µg). Tetracyclines; oxytetracycline (OT,30µg), tetracycline (T,30µg). Fosfomycin (FOS,50µg). The *E. coli* ATCC 25922 strain was used as a quality control strain.

Tolerance against the five heavy-metal ions including Hg, Cd, Cr, Pb, and Cu was examined by the broth dilution testing method (microdilution) as described in He et al. (2016). Metal chlorides $CuCl_2$, $CrCl_3$, $PbCl_2$ and $CdCl_2$ (Samchun, Seoul, Korea) were used to obtain Cu, Cr, Pb and Cd concentration series ranging between 3,200µg/mL and 6.25 µg/mL. $HgCl_2$ was dissolved to obtain a 400 to 0.78 µg/mL concentration series of Hg. *E. coli* K-12 (MG 1655) strain was used as the control strain and isolates that surpassed the MIC values of the control strain were considered as the resistant isolates.

Detection of antimicrobial resistance genes, class 1 integron, and heavy metal resistant genes

The presence of the antimicrobial resistance genes, class 1 integron gene cassettes, and heavy metal resistance genes were screened by the conventional PCR method. Primer sequences are presented in table 1. Each PCR reaction mixture was 30µL in final volume consisting 0.3µL of AmpOne Taq DNA polymer*A*se (GeneAll, Seoul, Korea), 3µL of dNTP mix, 3µL of 10×Taq polymerase buffer, 1µL of each forward and reverse primers, 1µL of template DNA and 21.7µL of PCR water. Amplified PCR products were visualized by gel electrophoresis on 1.5% (w/v) agarose gels. PCR thermocycler conditions for each reaction; initial denaturation of 94ºC for 2 min followed by a total of 35 cycles of amplification. Each cycle consisted of 94ºC denaturation for the 30s, annealing for 50s and 72ºC extension for 1 min.

Table 1 Oligonucleotide primers used to amplify antimicrobial resistance genes and integrons of *P. aeruginosa*.

Category	Gene		Sequence (5'-3')	Anne-ling temp (0C)	Ampli-con size (bp)	Refer-ence
Extended-spectrum β-lactamase	*blaTEM*	F	CATTTCCGTGTCGCCCTTATTC	58	1080	[1]
		R	CGTTCATCCATAGTTGCCTGAC			
	blaSHV	F	AGCCGCTTGAGCAAATTAAAC	58	795	[1]
		R	ATCCCGCAGATAAATCACCAC			
	blaCTX-M	F	CGCTTTGCGATGTGCAG	52	550	[1]
		R	ACCGCGATATCGTTGGT			
	blaOXA	F	GGCACCAGATTCAACTTTCAAG	55	564	[1]
		R	GACCCCAAGTTTCCTGTAAGTG			
Tetracycline resistance	*tetA*	F	GTAATTCTGAGCACTGTCGC	62	1000	[2]
		R	CTGCCTGGACAACATTGCTT			
	tetB	F	CTCAGTATTCCAAGCCTTTG	58	400	[2]
		R	CTAAGCACTTGTCTCCTGTT			
	tetE	F	GTGATGATGGCACTGGTCAT	62	1100	[2]
		R	CTCTGCTGTACATCGCTCTT			
Aminoglycoside resistance	*strA-strB*	F	TATCTGCGATTGGACCCTCTG	55	538	[3]
		R	CATTGCTCATCATTTGATCGGCT			
	aphAI-IAB	F	AAACGTCTTGCTCGA GGC	33	500	[4]
		R	CAAACCGTTATTCATTCGTGA			
	aac(3')-IIa	F	ATGGGCATC ATTCGCACA	55	749	[5]
		R	TCTCGGCTTGAACGAATTGT			
	aac(6')-Ib	F	TTGCGATGCTCTATGAGTGGCTA	55	482	[6]
		R	CTCGAATGCCTGGCGTGTTT			
Integrons	*intI1* (Class 1 integron integrase)	F	CTACCTCTCACTAGTGAGGGGCGG	58	485	[7]
		R	GGGCAGCAGCGAAGTCGAGGC			
	Class 1 integron	5'-CS	GGCATCCAAGCAGCAAG	56	variable	[8]
		3'-CS	AAGCAGACTTGACCTGA			
Heavy metal resistance genes	*CopA (Copper translocating ATPase)*	F	CGGTCTCTACGAATACCGCTTCAA	55	1300	[9]
		R	GAAATAGCTCATTGCCGAGGCGTT			
	CzcA (Cobalt/Zinc/Cadmium efflux protein)	F	GTTCACCTTGCTCTTCGCCATGTT	55	320	[9]
		R	ACAGGTTGCGGATGAAGGAGATCA			
	ChrR (Chromium resistance)	F	ATGTCTGATACGTTGAAAGTTGTTA	54	350	[10]
		R	CAGGCCTTCACCCGCTTA			
	merA (Mercuric reductase)	F	GTGCCGTCCAAGATCATGAT	57	933	[10]
		R	TAGCCYACRGTSGCSACYTG			

RESULTS

Species identification and antimicrobial resistance profile

A total of twenty *P. aeruginosa* isolates were isolated from the 40 pet Chinese stripe-necked turtles and examined for the presence of antimicrobial and heavy metal resistance determinants. Twenty of forty isolates showed typical colony growth on CN agar. The BLAST analysis results after 16S rRNA sequencing indicated a 99-100% match to *P. aeruginosa* sequences available in GenBank database which confirmed their identity as *P. aeruginosa*. Table 2 represents the antimicrobial resistance patterns and the MAR index values obtained according to the disc diffusion test. MAR indices of all *P. aeruginosa* isolates in this study was ≥ 0.2. The highest MAR index value was recorded as 0.45. Ampicillin and oxacillin resistance was observed in all isolates (Figure. 1). In addition to that, 14 (70%) isolate demonstrated amoxicillin resistance. Tetracycline and oxytetracycline resistance was observed in 85% and 90% of the isolates respectively. Resistance to streptomycin, imipenem, fosfomycin, kanamycin, gentamycin, tobramycin, and piperacillin resistance was detected in 60%, 40%, 15%, 50%, 20%, 10% and 5% of the isolates, respectively (Table 2).

Table 2 Antimicrobial resistance of *P. aeruginosa* isolated from CSN turtles

Isolate	Resisted antimicrobials[a]	intermediate resistance	MAR index
N01	OXO,AMO,T,AMP,KAN,STR	SXT	0.3
N03	OXO,OT,AMO,T,AMP,STR	SXT,KAN,IMP	0.3
N04	OXO,SXT,AMO,T,AMP,KAN,STR	PRL,CEFO	0.35
N05	IMI,OXO,OT,T,AMP		0.25
N07	IMI,OXO,OT,T,AMP	TOB,SXT,CEFO,STR	0.25
N12	IMI,OXO,OT,T,AMP,KAN,STR	AMO,SXT,GEN,	0.35
N15	IMI,TOB,OXO,CRO,OT,SXT,AMO,T,AMP	CEFO,STR	0.4
N20	OXO,CRO,OT,AMO,T,AMP,STR	KAN	0.35
N22	IMI,OXO,OT,AMO,T,AMP	-	0.3
N24	IMI,OXO,CRO,AMO,AMP,KAN,STR	SXT,SEFO	0.35
N25	IMI,OXO,OT,AMO,T,AMP	GEN,STR	0.3
N26	OXO,OT,SXT,AMO,T,AMP,STR	GEN	0.35
N28	OXO,OT,SXT,AMO,PRL,AMP,KAN,STR	IMI,GEN	0.4
N29	OXO,OT,SXT,AMO,T,AMP,KAN,GEN,STR		0.45
N30	OXO,OT,T,AMP	AMO,STR	0.2
N31	OXO,OT,SXT,AMO,T,AMP,KAN,GEN,STR	IMI	0.45
N32	OXO,OT,SXT,AMO,T,AMP,KAN,GEN,STR	IMI,CEFO	0.45
N33	OXO,CRO,OT,SXT,AMO,T,AMP,KAN,GEN,STR	IMI	0.5
N34	IMI,OXO,OT,SXT,AMO,T,AMP	KAN,STR	0.35
N35	IMI,TOB,OXO,OT,AMO,T,AMP,KAN	STR	0.4

[a] Resisted antimicrobial; AMP = ampicillin, STR = streptomycin, GEN= gentamycin, KAN = kanamycin, IMI = imipenem, CRO = ceftriaxone, OT = oxytetracycline, OXO= oxacillin, TOB= tobramycin, PRL= piperacillin, SXT= fosfomycin, CEFO= cefotaxime

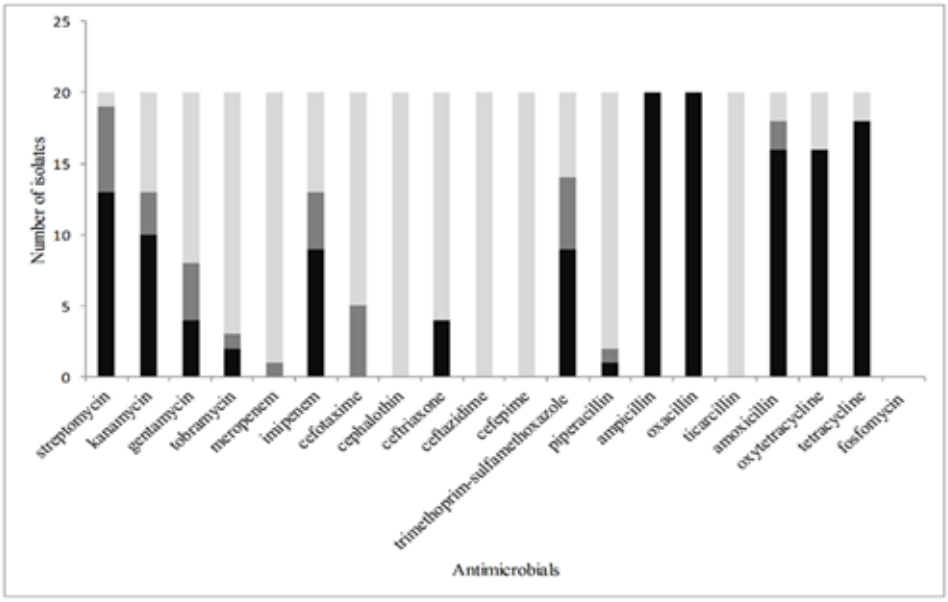

Figure 1 Antimicrobial susceptibility profile of *P. aeruginosa* isolated from pet Chinese stripe-necked turtles (Resistance,Intermediateresistance, Susceptible).

The Extended-spectrum beta-lactamases (ESBLs) encoding gene that frequently detected in this study was *blaSHV* (55%), followed by *blaTEM* (50%), *blaCTX* (10%) And *blaOXA* (5%) (Table 3). The genes, *aac*(6´)-*Ib* and *aphAI-IAB* were detected from 40% and 5% of the isolates respectively. Nine isolates could amplify class 1 integron integrase gene *intI1* and seven out of the nine isolates amplified class 1 integron gene cassette gene *aadA1*.

Minimum inhibitory concentration and heavy-metal resistance genes

All *P. aeruginosa* isolates demonstrated resistance to Cu and Cd (Table 3) in the broth dilution test. Hg, Cr, and Pb resistance were not observed. However, all tested heavy metal resistance genes were reported in different combinations. The heavy metal resistance genes, *CzcA* and *CopA* genes were detected in 65% and 30% of the isolates, respectively. *ChrR* and *merA* genes were reported in only 02(10%) and 01 (5%) isolates, respectively.

Table 3 Antimicrobial resistance genes, class 1 integron gene cassette and heavy metal resistance genes.

Isolate	Antimicrobial resistance gene	Class1 integron and gene cassette	Resisted heavy metals	Heavy metal resistance gene
N01	*blaSHV, blaCTX*		Cu, Cd	*CzcA*
N03	*blaSHV*		Cu, Cd	*ChrR*
N04	*blaSHV, BblaTEM*	*intl1,aadA1*	Cu, Cd	*CopA,CzcA*
N05			Cu, Cd	*ChrR*
N07		*intl1,aadA1*	Cu, Cd	*merA*
N12			Cu, Cd	*CopA,CzcA*
N15	*blaSHV*	*intl1,aadA1*	Cu, Cd	*CzcA*
N20	*blaSHV,blaTEM,blaCTX,blaOXA*		Cu, Cd	
N22	*aac(6')-Ib*		Cu, Cd	*CzcA*
N24	*blaTEM, aac(6')-Ib*	*intI1,aadA1*	Cu, Cd	*CzcA*
N25	*aac(6')-Ib*	*intl1,aadA1*	Cu, Cd	*CopA,CzcA*
N26	*blaTEM*		Cu, Cd	*CopA,CzcA*
N28			Cu, Cd	*CzcA*
N29			Cu, Cd	*CzcA*
N30	*blaTEM*		Cu, Cd	*CzcA*
N31	*blaSHV,blaTEM,aac(6')-Ib*	*intl1,aadA1*	Cu, Cd	
N32	*blaSHV,blaTEM,aac(6')-Ib*	*intl1,aadA1*	Cu, Cd	*CzcA*
N33	*blaTEM,aac(6')-Ib*	*aadA1*	Cu, Cd	*CzcA*
N34	*blaSHV,blaTEM,aac(6')-Ib*	*intl1*	Cu, Cd	*CopA*
N35	*blaSHV*	*intl1*	Cu, Cd	*CopA*

DISCUSSION

Antimicrobial resistance has been reporting since the introduction of antimicrobials to the world. However, now it has become one of the most important health problems as the bacteria have become resistant to treatments rapidly than ever we thought. Hence, not only studying frequently reported reservoirs of antimicrobial resistant determinants but also studying about poorly reported reservoirs is important in order to control the rate of spreading antimicrobial resistance. Particularly, pet animals in the domestic environment. Among the pet animals, turtles are unique reptiles that are gaining popularity as pets. CSN turtles are one of the most popular turtles in the Korean pet trade (Wendt and Heo, 2016). Turtles often carry many zoonotic pathogens, other than *Salmonella* spp., as part of their commensal gastrointestinal flora, including *Klebsiella* spp., *Aeromonas* spp., *Citrobacter* spp., and *Pseudomonas* spp. Despite this, CSN turtles are rarely studied for zoonotic diseases (Anderson, 2008). Therefore, studying the antimicrobial resistance of *P. aeruginosa* isolated from CSN turtles is important in terms of controlling antimicrobial resistance. A total of 20 *P. aeruginosa* isolates were identified from 40 turtles up to species level by sequencing with the 16S rRNA genes.

Multiple antimicrobial resistance (MAR) indexing has been proven to be an effective and valid criterion of bacteria source tracking. Bacteria that have MAR index values ≥ 0.2 indicates the origin from high-risk sources of contamination where antimicrobials are often used (Krumperman, 1983; Osundiya et al., 2013). Interestingly, MAR indices of all *P. aeruginosa* isolates

in this study was ≥ 0.2. The highest MAR index value was recorded as 0.45. Multidrug-resistant *P. aeruginosa* has been reported worldwide in hospital settings. However, Radó et al., (2017) have studied and reported that non-clinical multidrug-resistant *P. aeruginosa* isolates can also be a potential risk of public health.

Eight categories of antimicrobials are generally used to treat *P. aeruginosa* infections including penicillin with β-lactamase inhibitors, aminoglycosides (tobramycin, gentamicin, amikacin, netilmicin), carbapenems (meropenem, imipenem), cephalosporins (ceftazidime, cefepime), fluoroquinolones (ciprofloxacin, levofloxacin), monobactams (aztreonam), fosfomycin and polymyxins (Bassetti et al., 2018). Among them, imipenem, fosfomycin, gentamycin, tobramycin, and piperacillin resistance were detected in 40%, 15%, 20%, 10% and 5% of the isolates, respectively (Table 2). Further, several isolates demonstrated intermediate resistance to these antimicrobials. These are in the suggested groups of antimicrobial agents by the Clinical and Laboratory Standards Institute (CLSI) that should be considered for routine testing and reporting. As a well-known antimicrobial-resistant bacterium in hospital environments, the demonstrated antimicrobial resistance to front line antimicrobials by pet turtle borne *P. aeruginosa* in this study reveals the capability to become a troublesome pathogen in the domestic environment.

Extended-spectrum β-lactamases (ESBLs) producing *P. aeruginosa* demonstrates a high degree of resistance to most of the β-lactam antimicrobials including, cephalosporins and penicillins. We could detect 100% resistance to ampicillin and oxacillin (Figure: 1). In addition to that, 14 (70%) isolate demonstrated amoxicillin resistance. Almost all *P. aeruginosa* isolates intrinsically resistant to these antimicrobials. However, there can be some susceptible strains. Therefore testing resistance is necessary to optimize treatments (CLSI, 2014). Although ceftriaxone and trimethoprim-sulfamethoxazole resistance is common in almost all representatives of *P. aeruginosa*, 100% and 60% of the isolates were susceptible in this study, respectively.

The ESBL genes that predominated in this study was *blaSHV* (55%), followed by *blaTEM* (50%), *blaCTX* (10%) And *blaOXA* (5%) (Table 3). *blaSHV* and *blaTEM* are the most frequently reported ESBLs in Korea (Kim et al. 2005). These ESBL genes harboring *P. aeruginosa* isolates have been reported from different parts of the world. This early detection of ESBLs would be essential to reduce the spreading rate of multidrug-resistant *P. aeruginosa* in the domestic environment.

Tetracycline and oxytetracycline resistance was observed in 85% and 90% of the isolates respectively. In contrast, *tetB* gene was detected only in one isolate. Tetracyclines are not very effective against *P. aeruginosa*. as most of the *P. aeruginosa* isolates show intrinsic resistance to tetracyclines (Morita et al., 2013).

Enzyme mediated modification of aminoglycosides by aminoglycoside-phosphotransferases (APH), aminoglycoside-adenyltransferase (AAD) and aminoglycoside-acetyltransferases (AAC) is the most reported aminoglycoside resistance mechanism in *P. aeruginosa*. Genes that encode these enzymes can be found on mobile genetic elements (Teixeira et al., 2016). The most frequent aminoglycoside resistance gene in this study was *aac*(6′)-*Ib*, detected in 8

isolates. One isolate was positive for the *aphAI-IAB* gene. *aac*(6′)-*Ib* is one of the most frequently identified aminoglycoside resistance genes in Korea (Strateva and Yordanov 2009). Seven isolates could amplify gene cassette gene *aadA1* that confers streptomycin and spectinomycin resistance. *aac*(6′)-*Ib* and *aadA1* genes were also shown to be simultaneously present in 6 isolates. Streptomycin, kanamycin and gentamycin resistance observed in 12, 10 and 4 isolates, respectively. We found a discrepancy between aminoglycoside resistant gene profile and phenotypic profile. This implies that other aminoglycoside resistant genes, not examined here, may be present in our isolates, as reported elsewhere (Vaziri et al., 2011). Furthermore, the presence of antimicrobial resistance genes but not phenotypic resistance can occur due to the presence of non-functional protein-producing mutations and the lack of promoters (Mella et al., 2004).

Nine *P. aeruginosa* isolates in this study amplified class1 integron integrase gene *intI1*. This gene is one of the three core features in class1 integron and is linked to genes conferring resistance to antimicrobials and heavy metals (Gillings et al., 2015). Seven *intI1*-positive isolates harbored gene cassettes gene *aadA1*. The number of antimicrobial resistance genes detected in these 7 isolates was high. Zheng et al., (2020) have identified a significant positive relationship between the overall abundance of antimicrobial resistance genes and the *intI1* gene. Hence, *intI1* present *P. aeruginosa* in this study could be able to acquire more antimicrobial resistant genes.

Heavy metals are among the well-characterized groups of antimicrobial resistant-driving chemicals (Singer et al., 2016). *P. aeruginosa* and *E. coli* are the most studied bacteria for the co-occurrence of heavy metal and antimicrobial resistance (Nguyen et al., 2019). In our study, all *P. aeruginosa* isolates demonstrated resistance Cu and Cd (Table 3). Hg, Cr, and Pb resistance was not observed. *P. aeruginosa* is a well-known bacterium for tolerance to Cd and Cu (Bédard et al., 2014; Chellaiah, 2018). Moreover, Chellaiah (2018) has proposed Cd resistant *P. aeruginosa* strains as multidrug-resistant strains. In agreement with previous reports, all isolates in this study scored MAR indices ≥0.2. Tolerability to Cd, Co, Cu, and Zn render *P. aeruginosa* resistant to antimicrobials. Especially, to carbapenems (Dieppois et al., 2012). However, imipenem resistance was the only carbapenem resistance we could detect in this study.

Although our isolates have a strong tolerance to Cd and Cu, *CzcA* and *CopA* genes were observed only in 13 and 6 isolates respectively. Cd and Cu resistance in *P. aeruginosa* is a complex process and still being studied. *CzcA* and *CopA* genes in *P. aeruginosa* have been identified as regulators involved in the control of virulence and antimicrobial resistance. Even though they are isolated from non-clinical sources (Dieppois et al., 2012; Petitjean et al., 2017). Thus, further studies required to study Cd and Cu resistance in pet turtle borne *P. aeruginosa* to evaluate its possible contributions to spread pathogenic *P. aeruginosa* strains in the domestic environment.

Cr and Hg resistance was not observed in this study. However, *ChrR* and *merA* genes were reported in 2 and 1 isolates, respectively. Microorganisms bearing the *ChrR* gene cannot express Cr resistance always due to the highly variable resistance capacities to different Cr concentrations (Viti et al., 2014). Regarding the Hg resistance, it has identified that the relatively low level of Hg resistance when the presence of the *merA* gene alone (Naguib et al., 2018). However, Hg resistant genes are usually located on transposons and plasmids which carry antimicrobial resistance genes as well (Mirzaei et al., 2013).

CONCLUSIONS

This study detected the high prevalence of beta-lactam resistance genes and the Cobalt-zinc cadmium resistance *CzcA* gene from the isolated *Pseudomonas aeruginosa*, and they are multidrug-resistant strains. Moreover, all isolates demonstrated resistance to Cu and Cd. Therefore, antimicrobial and heavy metal resistance properties in *P. aeruginosa* isolated from pet Chinese stripe-necked turtles are notable. Other than the presence of well-known pet turtle borne pathogen *Salmonella* spp., pet Chinese stripe-necked turtles may seem harmless, giving a false sense that they are safe to rare closely. However, our findings reveal the presence of antimicrobial-resistant properties in *P. aeruginosa* isolated from Pet Chinese stripe-necked turtles that can cause problems in upon infections. Pet turtle born *P. aeruginosa* can be a potential risk to public health and cannot be excluded as a non-nosocomial source of infections. Further studies required to confirm the mobility and acquisition of more resistance determinants as well as colonization in domestic environments in order to increase public awareness.

ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sector.

AUTHOR CONTRIBUTIONS

M.V.K.S. Wickramanayake and L.A.D.S. De Silva contributed equally to this work

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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How to cite this article;

M.V.K.S. Wickramanayake, L.A.D.S. De Silva and Gang-Joon Heo. Pseudomonas aeruginosa from pet Chinese stripe-necked turtles (*Ocadia sinensis*) demonstrating antimicrobial and heavy metal resistance. Veterinary Integrative Sciences. 2022; 20(3): 761- 773.