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Identification and antibiotic resistance profiling of bacterial isolates from septicaemic soft-shelled turtles (*Pelodiscus sinensis*)

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ABSTRACT: The present study sought to identify pathogens associated with septicaemia in the Chinese soft-shelled turtle (*Pelodiscus sinensis*) and to characterise antibiotic resistance in these pathogens. Twenty-three isolates recovered from the livers of diseased soft-shelled turtles were genetically identified as *Aeromonas hydrophila* (*n* = 8), *A. veronii* (*n* = 3), *Citrobacter freundii* (*n* = 4), *Morganella morganii* (*n* = 3), *Edwardsiella tarda* (*n* = 2), *Wohlfahrtiimonas chitiniclastica* (*n* = 1), *Chryseobacterium* sp. (*n* = 1), and *Comamonas* sp. (*n* = 1). Most isolates (*n* = 21) were resistant to ampicillin whereas a low percentage of isolates was susceptible to aminoglycosides (amikacin, gentamicin, and tobramycin). PCR assays and sequence analysis revealed the presence of the *qnrS2* and bla_{TEM} antibiotic resistance genes in all isolates. The bla_{DHA-1}, $bla_{CTX-M-14}$ and bla_{CMY-2} genes were harboured by 17.4% (*n* = 4), 13.5% (*n* = 3) and 8.7% (*n* = 2) of the strains, respectively. One or more tetracycline resistance genes were detected in 60.9% (*n* = 14) of the isolates. Four isolates (17.4%) harboured single or multiple class 1 integron cassettes. Collectively, a variety of bacterial pathogens were involved in the occurrence of septicaemia in Chinese soft-shelled turtles and most of the isolates had multi-antibiotic resistant phenotypes. To our knowledge, the present report is the first to identify *W. chitiniclastica* and *Comamonas* sp. as causes of septicaemia in soft-shelled turtles and the first to identify *Aeromonas* spp. with $bla_{CTX-M-14}$ and bla_{DHA-1} resistance genes.

Keywords: pet; ulcer disease; liver; lesions; septicaemia; antibiotic resistance

The Chinese soft-shelled turtle (*Pelodiscus sinensis*) is a reptile that lives in fresh water. In addition to its popularity as an indoor pet, the species is a traditional nutrient-rich food in Asian countries (Feng et al. 1996; Yin et al. 2005), which has led to commercial aquaculture of these animals in land-based tanks or ponds in various countries including China and Japan. In Korea, they are farmed to a lesser extent than other aquatic animals. Over the past two decades, the soft-shelled turtle has been one of the most intensively cultured fresh-water animals. However, infectious diseases that cause problems such as skin ulcers, shell necrosis, and septicaemia continue to threaten soft-shelled turtle farms.

In turtles, septicaemia manifests as various clinical symptoms including anorexia, lethargy, shell necrosis, and skin ulcers. Another significant *post-mortem* observation is liver necrosis (Kohler 2006). *Citrobacter freundii* is a major pathogen responsible for septicaemic cutaneous ulcerative disease in turtles (Kohler 2006). In China, *Aeromonas* spp., *C. freundii*, and *Edwardsiella tarda* have frequently been reported as aetiological agents of septicaemia in the Chinese soft-shelled turtle (Hu

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et al. 2010; Chen et al. 2013a; Chen et al. 2013b). C*hryseobacterium* spp. and *Morganella morganii* can also be isolated from turtles with shell disease, if only rarely (Hernandez-Divers et al. 2009). In the absence of vaccines against specific bacteria, antibiotic treatment is the best way to control these pathogens in aquaculture. Most previous studies on the antimicrobial susceptibilities of turtle isolates have focused on the bacterial flora of wild and captive turtles as bio-indicators for polluted effluents (Al-Bahry et al. 2009; Foti et al. 2009; Al-Bahry et al. 2012; Wheeler et al. 2012). In addition, *Salmonella* spp. in pet turtles have been demonstrated to be a risk factor for human salmonellosis (Seepersadsingh and Adesiyun 2003; Diaz et al. 2006; Chen et al. 2010; Guerra et al. 2010). To date, the prevalence of antibiotic resistance genes has been examined in only a few studies in pathogenic bacteria isolated from farmed soft-shelled turtles.

To our knowledge, no studies have focused on the aetiological agents responsible for the occurrence of infectious bacterial diseases in commercial Chinese soft-shelled turtles farmed in Korea. Furthermore, little information has been obtained with regard to antibiotic resistance and its genetic determinants in this reptile. Data on the antibiotic resistance of pathogens are of crucial importance for efforts directed towards the prevention and control of infectious diseases in soft-shelled-turtle farms. Therefore, the aims of the present study were to identify the bacterial pathogens associated with septicaemia in the soft-shelled turtle and to characterise the antibiotic resistance of these pathogens. Furthermore, we investigated the prevalence of the following antibiotic resistance determinants: β-lactamase encoding genes, tet, plasmid-mediated qnr and the class 1 integron cassette.

MATERIAL AND METHODS

Isolation. Bacteria were isolated from the liver lesions of 23 farm-raised Chinese soft-shelled turtles (*P. sinensis*) with various clinical symptoms (lethargy, slow growth, and skin and shell ulceration) in Korea. The bacterial isolations were performed by streaking liver samples onto tryptic soy agar (TSA) agar plates followed by incubation overnight at 27 °C. The isolated colonies were then subcultured on TSA plates to obtain pure colonies. A total of 23 strains were obtained and stored at –70 °C in CryoCare Bacteria Preservers (Key Scientific Products, Stampford, USA) until required for further laboratory procedures.

Identification. The stored bacteria were incubated in tryptic soy broth overnight at 27 °C. The bacterial suspensions were processed using an AccuPrep® genomic DNA extraction kit (Bioneer, Daejeon, Korea) for the purification of bacterial DNA. The concentration of purified DNA was determined with the aid of an Epoch spectrophotometer system (Biotek, USA). Bacterial identifications were performed using standard primer sets (27F: 5'-AGAGTTTGATCMTGGCTCAG-3', 1492R: 5'-TACGGYTACCTTGTTACGACTT-3') for 16S rDNA sequencing (Macrogen Service Center, Daejeon, Korea). Partial gyrB sequencing was conducted to verify 11 *Aeromonas* strains to the species level, according to our previous study (Yi et al. 2013) using the same primer sets listed in Table 1.

Disc-diffusion assays. The antimicrobial susceptibility profile for each of the strains was investigated using the disc-diffusion method with 16 different antimicrobial agents: amikacin (AN, 30 mg), gentamicin (GM, 10 mg), tobramycin (NN, 10 mg), enrofloxacin (ENR, 5 mg), norfloxacin (NOR, 10 mg), oxolinic acid (OA, 2 mg), sulfamethoxazole/trimethoprim (Sxt, 300 mg + 5 mg), tetracycline (25 mg), amoxicillin/clavulanic acid (AMC, 30 mg), ampicillin (AM, 10 mg), piperacillin (PIP, 100 IU), cefotaxime (CTX, 30 mg), cephalothin (CF, 30 mg), cefaclor (CEC, 5 mg), imipenem (IMP, 10 mg), and chloramphenicol (C, 30 mg). The strains were recovered from the freezer with growth on TSA and then tested on Muller-Hinton agar plates. Various antimicrobial discs were then applied to the cultures. The inhibition zones were measured after incubation for 18 h at 27 °C. The resistance of the strains to the antimicrobials was determined according to the manufacturer's instructions and the M100-S17 document of the Clinical and Laboratory Standards Institute.

Detection of antibiotic resistance genes. All of the strains were tested by PCR assays to detect the genetic determinants associated with resistance to tetracycline, quinolones, and β-lactams, in addition to the class 1 integron gene cassette encoding resistance to various antimicrobials. The primer sets and PCR conditions are summarised in Table 1. The assays were carried out in 20 ml AccuPower® PCR premix (Bioneer) containing 1 ml of each forward and reverse primer (10mM) and 1 ml of bacterial genomic

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Table 1. PCR primers used in this study

PCR	Target	Primer pair	Sequence (5'-3')	Annealing temperature (°C)	Amplicon size (bp)	Reference
S	*gyrB*	gyrB-3F gyrB-14R	TCCGGCGGTCTGCACGGCGT TTGTCCGGGTTGTACTCGTC	68	1100	Martinez-Murcia et al. (2011)
S	class 1 integron	5'-CS 3'-CS	GGCATCCAAGCAGCAAG AAGCAGACTTGACCTGA	64	VR	Lee et al. (2008)
M-1	$bla_{CTX-M-1}$ group	CTXGp1-F CTXGp1-R	TTAGGAARTGTGCCGCTGYA CGATATCGTTGGTGGTRCCAT	68	688	Dallenne et al. (2010)
	$bla_{CTX-M-2}$ group	CTXGp2-F CTXGp2-R	CGTTAACGGCACGATGAC CGATATCGTTGGTGGTRCCAT	68	404	
	$bla_{CTX-M-9}$ group	CTXGp9-F CTXGp9-R	TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG	68	561	
M-2	bla_{TEM}	TEM-F TEM-R	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	60	800	Dallenne et al. (2010)
	bla_{SHV}	SHV-F SHV-R	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC	60	713	
	$bla_{OXA-A-like}$ variants	OXAA-F OXAA-R	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG	60	564	
M-3	*qnrA*	qnrA-F qnrA-R	AGAGGATTTCTCACGCCAGG TGCCAGGCACAGATCTTGAC	60	580	Cattoir et al. (2007)
	qnrB	qnrB-F qnrB-R	GGAATCGAAATTCGCCACTG TTTGCCGTCCGCCAGTCGAA	60	264	
	qnrS	qnrS-F qnrS-R	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	60	428	
M-4	*tetA*	tetA-F tetA-R	GTAATTCTGAGCACTGTCGC CTGCCTGGACAACATTGCTT	62	1000	Akinbowale et al. (2007)
	tetE	tetE-F tetE-R	GTGATGATGGCACTGGTCAT CTCTGCTGTACATCGCTCTT	62	1200	
M-5	*tetB*	tetB-F tetB-R	CTCAGTATTCCAAGCCTTTG CTAAGCACTTGTCTCCTGTT	57	400	
	tetD	tetD-F tetD-R	ATTACACTGCTGGACGCGAT CTGATCAGCAGACAGATTGC	57	1100	
S	*tetC*	tetC-F tetC-R	TCTAACAATGCGCTCATCGT GGTTGAAGGCTCTCAAGGGC	62	588	
S	*tetM*	tetM-F tetM-R	GTTAAATAGTGTTCTTGGAG CTAAGATATGGCTCTAACAA	55	600	

S = single PCR, M = multiplex PCR, VR = variable

DNA (30–40 ng). The amplicons for each gene were resolved by electrophoresis on 1.5% agarose/TBE gels including RedSafe (iNtRON Biotechnology) and visualized under UV light. The class 1 integron amplicons were purified using the AccuPower® gel purification kit (Bioneer) and sequenced directly using 5'-CS and 3'-CS primers (Macrogen Service Center, Korea). The class 1 integron gene cassette homology searches were performed using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov/BLAST).

RESULTS

Twenty-three isolates were independently recovered from the livers of diseased Chinese soft-

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shelled turtles with skin/shell ulcers. Based on 16S rDNA sequences, the 23 isolates were divided into eight different species belonging to six families: *A. hydrophila* (*n* = 8), *A. veronii* (*n* = 3), *M. morganii* (*n* = 3), *C. freundii* (*n* = 4), *Wohlfahrtiimonas chitiniclastica* (*n* = 1), *Chryseobacterium* spp. (*n* = 1), *Comamonas* spp. (*n* = 1), and *E. tarda* (*n* = 2) (Table 2). There are many reports of strong similarities between the 16S rDNA sequences of *Aeromonas* species (Yanez et al. 2003); therefore, phylogenetic analysis using partial gyrB sequences was applied to verify the species-level identification of the 11 *Aeromonas* isolates. The results corresponded to those obtained using the 16S rDNA method for those same isolates.

The antibiotic resistance of the isolates was determined using the disc diffusion test (Table 2). Resistance phenotypes of all the isolates to 16 different antibiotics and determinants are summarised in Table 3. A high percentage of the isolates were resistant to ampicillin (95.7%), cefaclor (78.3%) and cephalothin (73.9%), whereas a low percentage was resistant to aminoglycosides (13.1%). The trend of resistance according to major bacterial family was found as follows: AM (100%) > CF = CEC = TE (72.7%) > IPM = PIP (63.6%) > OA = Sxt (27.3%) > AMC = C = CTX (18.2%) for the Aeromonasceae and AM (88.9%) > CEC (77.8%) > CF = TE = AMC (66.7%) > PIP = C = CTX (55.6%) > ENR = NOR = OA = Sxt (44.4%) > IPM (22.2%) > GM = NN (11.1%) for the Enterobacteriaceae. Twenty-one of the 23 isolates (91.3%) exhibited greater than intermediate resistance to three or more antimicrobial agents (the exceptions were two *E. tarda* isolates).

Table 2. Antibiotic resistance of the isolates

Isolate	AM		AMC		CEC		CF		CTX		PIP		IPM		AN		GM		NN		ENR		NOR		OA		C		Sxt		TE	
	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I
SST001	0	R	18	S	10	R	0	R	29	S	21	S	9	R	24	S	20	S	20	S	30	S	26	S	26	S	30	S	26	S	26	S
SST002	0	R	21	S	17	I	18	S	29	S	25	S	19	S	27	S	20	S	18	S	25	S	20	S	0	R	19	S	0	R	21	S
SST003	0	R	12	R	0	R	30	S	12	R	0	R	0	R	22	S	18	S	17	S	0	R	12	R	0	R	0	R	0	R	0	R
SST004	0	R	19	S	20	S	21	S	33	S	20	I	0	R	22	S	21	S	20	S	24	S	21	S	0	R	27	S	26	S	0	R
SST005	0	R	21	S	0	R	0	R	22	I	17	R	14	I	24	S	21	S	22	S	29	S	26	S	23	S	26	S	26	S	11	R
SST006	0	R	19	S	12	R	12	R	27	S	20	I	12	R	22	S	22	S	23	S	26	S	26	S	24	S	27	S	25	S	10	R
SST007	0	R	15	I	0	R	0	R	27	S	25	S	20	S	21	S	16	S	20	S	23	S	25	S	20	S	0	R	25	S	20	S
SST008	0	R	20	S	12	R	11	R	29	S	21	S	16	I	23	S	21	S	23	S	23	S	24	S	22	S	30	S	26	S	12	R
SST009	0	R	12	R	0	R	0	R	22	I	18	I	19	S	17	S	17	S	16	S	0	R	12	R	0	R	19	S	0	R	0	R
SST010	0	R	13	R	0	R	0	R	22	I	19	I	19	S	20	S	20	S	20	S	29	S	26	S	24	S	17	I	25	S	0	R
SST011	0	R	13	R	0	R	0	R	17	I	17	R	24	S	21	S	15	S	18	S	0	R	10	R	0	R	0	R	0	R	0	R
SST012	0	R	16	I	0	R	0	R	0	R	13	R	15	I	16	I	15	S	18	S	22	S	25	S	19	S	28	S	22	S	25	S
SST013	0	R	19	S	10	R	0	R	26	S	19	I	11	R	22	S	21	S	21	S	24	S	24	S	21	S	24	S	27	S	10	R
SST014	0	R	20	S	18	S	14	R	30	S	19	I	18	S	20	S	22	S	19	S	24	S	25	S	25	S	26	S	22	S	25	S
SST015	0	R	19	S	10	R	0	R	26	S	21	S	15	I	24	S	20	S	18	S	30	S	27	S	24	S	25	S	0	R	15	R
SST016	0	R	18	S	20	S	22	S	28	S	25	S	12	R	22	S	19	S	20	S	31	S	29	S	31	S	27	S	32	S	11	R
SST017	0	R	12	R	0	R	11	R	23	S	10	R	22	S	20	S	15	S	16	S	0	R	12	R	0	R	0	R	0	R	10	R
SST018	0	R	16	I	0	R	0	R	0	R	14	R	17	S	11	R	0	S	0	S	33	S	22	S	28	S	10	R	26	S	18	I
SST019	0	R	18	S	13	R	0	R	0	R	16	R	16	I	11	R	0	S	0	S	24	S	12	R	22	S	12	R	24	S	11	R
SST020	0	R	23	S	0	R	0	R	19	I	21	S	25	S	20	S	16	S	16	S	29	S	23	S	20	S	28	S	29	S	26	S
SST021	21	S	25	S	24	S	22	S	28	S	22	S	25	S	23	S	16	S	15	I	31	S	28	S	29	S	24	S	27	S	25	S
SST022	16	I	22	S	25	S	20	S	31	S	18	S	22	S	18	S	12	R	16	S	28	S	27	S	27	S	27	S	27	S	21	S
SST023	0	R	11	R	0	R	0	R	21	I	13	R	24	S	23	S	16	S	20	S	0	R	12	R	0	R	9	R	0	R	10	R

AM = ampicillin, AMC = amoxicillin/clavulanic acid, AN = amikacin, C = chloramphenicol, CEC = cefaclor, CF = cephalothin, CTX = cefotaxime, D = diameter (mm), ENR = enrofloxacin, GM = gentamicin, I = intermediate, IMP = imipenem, NN = tobramycin, NOR = norfloxacin, OA = oxolinic acid, PIP = piperacillin, R = resistance, S = susceptible, Sxt = sulfamethoxazole/trimethoprim, TE = tetracycline

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Table 3. Characterisation of the 23 strains isolated from Chinese soft-shelled turtles according to resistance phenotypes and genetic determinants, including class 1 integron gene cassettes

Identification	Isolates	Resistance phenotype	Resistance genes	Class 1 integron gene cassette
A. hydrophila	SST001	AM, IPM, CF, CEC	*qnrS2*, $bla_{TEM-171}$	
	SST002	OA, Sxt, AM, CEC(I)	*qnrS2*, $bla_{TEM-171}$, *tetC*	*dfrA12-orfF-aadA2*, *catB3-aadA1*
	SST005	TE, AM, IPM, PIP, CTX(I), CF,CEC	*qnrS2*, $bla_{TEM-171}$, *tetA*, $bla_{CTX-M-14}$	
	SST006	TE, AM, IPM, PIP(I), CF, CEC	*qnrS2*, $bla_{TEM-171}$, *tetA*	
	SST013	TE, AM, IPM, PIP(I), CF, CEC	*qnrS2*, $bla_{TEM-171}$, *tetA*	
	SST014	AM, PIP(I), CF	*qnrS2*, $bla_{TEM-171}$	
	SST015	Sxt, TE, AM, IPM(I), CF, CEC	*qnrS2*, $bla_{TEM-171}$, *tetA*	
	SST018	AN, TE(I), AmC(I), AM, PIP, C, CTX, CF, CEC	*qnrS2*, $bla_{TEM-171}$	
A. veronii	SST004	OA, TE, AM, IPM, PIP(I)	*qnrS2*, $bla_{TEM-171}$, *tetA*	
	SST016	TE, AM, IPM	*qnrS2*, $bla_{TEM-171}$	
	SST017	ENR, NOR, OA, Sxt, TE, AmC, AM, PIP, C, CF, CEC	*qnrS2*, bla_{TEM-2}, bla_{DHA-1}, *tetA*, *tetB*	
C. freundii	SST007	AmC(I), AM, C, CF, CEC	*qnrS2*, $bla_{TEM-171}$	
	SST008	TE, AM, IPM(I), CF, CEC	*qnrS2*, bla_{TEM-2}, *tetD*, bla_{CMY-2}	
	SST009	ENR, NOR, OA, Sxt, TE, AmC, AM, PIP(I), CTX(I), CF, CEC	*qnrS2*, *qnrB14*, $bla_{TEM-171}$, bla_{CMY-2}, *tetA*	dfrA1-aadA1
	SST010	TE, AmC, AM, PIP(I), C(I), CTX(I), CF, CEC	*qnrS2*, *qnrB1*, $bla_{TEM-171}$, $bla_{CTX-M-14}$, *tetB*	
M. morganii	SST003	ENR, NOR, OA, Sxt, TE, AmC, AM, IPM, PIP, C, CTX, CEC	*qnrS2*, $bla_{TEM-171}$, *tetB*, bla_{DHA-1}	bla_{PSE-1}-*aadA2*, *aadB-catB3*
	SST011	ENR, NOR, OA, Sxt, TE, AmC, AM, PIP, C, CTX(I), CF, CEC	*qnrS2*, $bla_{TEM-171}$, bla_{DHA-1} *tetB*	bla_{PSE-1}-*aadA2*, *aadB-catB3*
	SST023	ENR, NOR, OA, Sxt, TE, AmC, AM, PIP, C, CTX, CF, CEC	*qnrS2*, $bla_{TEM-171}$, bla_{DHA-1}, *tetB*	
E. tarda	SST021	NN	*qnrS2*, $bla_{TEM-171}$	
	SST022	GM, AM(I)	*qnrS2*, $bla_{TEM-171}$, *tetA*	
Chryseobacterium sp.	SST019	AN, NOR, TE, AM, IPM, PIP, C, CTX, CF, CEC	*qnrS2*, $bla_{TEM-171}$	
Comamonas sp.	SST020	AM, CTX(I), CF, CEC	*qnrS2*, $bla_{TEM-171}$	
W. chitiniclastica	SST012	AN(I), AmC(I), AM, IPM(I), PIP, CTX, CF, CEC	*qnrS2*, $bla_{TEM-171}$, $bla_{CTX-M-14}$	

AmC = amoxicillin/clavulanic acid, AN = amikacin, AN = ampicillin, C = chloramphenicol, CEC = cefaclor, CF = cephalothin, CTX = cefotaxime, ENR = enrofloxacin, GM = gentamicin, I = interpretation according to CLSI M100-S17, IPM = imipenem, NN = tobramycin, NOR = norfloxacin, OA = oxolinic acid, PIP = piperacillin, Sxt = sulfamethoxazole/trimethoprim, TE = tetracycline

Multiple resistance to more than 10 antibiotics was observed in the *A. veronii* SST017, *C. freundii* SST009, *M. morganii* SST011, SST023, and SST003 and *Chryseobacterium* sp. SST019 isolates.

All isolates were subjected to PCR amplification and sequence analysis to identify the antimicrobial resistance determinants β-lactamase (*bla*), plasmid-mediated quinolone resistance (*qnr*), tetracycline resistance (*tet*), and class 1 integron (*intl1*) genes. The $bla_{TEM-171}$ and bla_{TEM-2} genes were detected in 21 and two isolates, respectively. The 14 isolates carrying only the $bla_{TEM-171}$ gene varied in resistance

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patterns to β-lactams. In addition, *E. tarda* isolates exhibited below intermediate resistance to ampicillin. On the other hand, nine of the isolates that harboured bla_{TEM} also carried $bla_{CTX\text{-}M\text{-}14}$ (*A. hydrophila* SST005, *C. freundii* SST010 and *W. chitiniclastic*a SST012), $bla_{DHA\text{-}1}$ (*A. veronii* SST017, *M. morganii* SST003, SST011 and SST023), or $bla_{CMY\text{-}2}$ (*C. freundii* SST008 and SST009). The *qnrS2* gene was detected in all isolates investigated in the present study, of which *C. freundii* SST009 and SST010 simultaneously carried *qnrB14* and *qnrB1*, respectively. The *tet* genes were detected in 14 isolates and the genes detected included *tetA* (8/14), *tetB* (5/14), *tetC* (1/14), and *tetD* (1/14). In *Aeromonas* isolates, *tetA* (6/11) was the most common *tet* determinant whereas *tetC* was detected in only *A. hydrophila* SST002. In addition, the simultaneous detection of *tetA* and *tetB* was observed in only *A. veronii* SST017. In the case of enteric bacteria, all the isolates possessed one of three *tet* determinants, *tetA*, *tetB* or *tetD*. The class 1 integron gene cassette was detected in the following four isolates: *dfrA12-orfF-aadA2* and *catB3-aadA1* in *A. hydrophila* SST002, *dfrA1-aadA1* in *C. freundii* SST009 and *blaPSE-1-aadA2* and *aadB-catB3* in *M. morganii* SST003 and SST011.

DISCUSSION

In the present study, most of the identified bacterial species have been reported as infectious agents responsible for septicaemia in chelonia (Oros et al. 2005; Hernandez-Divers et al. 2009; Hu et al. 2010). *C. freundii* is known to be the aetiological agent of septicaemic cutaneous ulcerative disease (SCUD), which is accompanied by liver necrosis (Kohler 2006). *Aeromonas* spp. have frequently been isolated from the liver or kidney of soft-shelled turtles with various clinical manifestations, such as soft shell and white abdominal shell (Chen et al. 2013a; Chen et al. 2013b). In addition, *M. morganii* and *C. indologenes* have been suggested to be infectious agents associated with shell necrosis in map turtles (*Graptemys* spp.; Hernandez-Divers et al. 2009). *E. tarda* and *C. freundii* have been isolated from clinical samples of soft-shelled turtles with fulminant septicaemia (Hu et al. 2010). In agreement with previous studies (Kohler 2006; Hernandez-Divers et al. 2009; Hu et al. 2010; Chen et al. 2013a; Chen et al. 2013b), we identified aeromonads and enteric bacteria as the major pathogens associated with septicaemia and skin/shell diseases in soft-shelled turtles. However, to the best of our knowledge, there is no information regarding infection with *W. chitiniclastica* and *Comamonas* spp. in chelonians. In addition, infections with both bacterial isolates have been reported only rarely in animals, including humans (Rebaudet et al. 2009; Almuzara et al. 2011; Nseir et al. 2011; Farshad et al. 2012). Moreover, *W. chitiniclastica* has yet to be recorded at all in Korea.

The literature data regarding the antimicrobial resistance patterns according to bacterial species and isolate sources are inconsistent (Foti et al. 2009; Chen et al. 2010; Al-Bahry et al. 2011; Al-Bahry et al. 2012; Aravena-Roman et al. 2012; Jang et al. 2013). In contrast to these previous reports, our major findings include higher resistance to cefotaxime and imipenem among enteric bacteria and aeromonads, respectively. These two antibiotics have not been approved for use in aquatic animals worldwide; therefore, resistance to these antibiotics has rarely been reported among bacterial isolates from aquatic animals, including turtles. The reason why the present isolates are highly resistant to both antibiotics remains to be established. Further studies are therefore warranted to examine whether the aforementioned antibiotic resistance is the result of contamination by polluted effluent.

Many studies have indicated that the aquatic environment is a vehicle for the spread of antibiotic-resistant bacteria and resistance genes (Jang et al. 2013; Marti et al. 2014). The present data further support this idea, and show a high prevalence and diversity of genetic determinants among the present isolates recovered from freshwater turtle aquaculture. The 14 isolates described here that harboured only the $bla_{TEM\text{-}171}$ gene varied in resistance patterns to β-lactams. These results might be due to varying levels of enzyme produced in different bacterial species and/or isolates, and indeed previous findings demonstrated that the degree of resistance to β-lactams depends on the amount of TEM or SHV enzyme produced among isolates (Wu et al. 1994; Livermore 1995). In addition, a high prevalence of the $bla_{TEM\text{-}171}$ gene was reported in only one previous study with *E. coli* isolates collected from a river in Korea (Jang et al. 2013). Although determining the prevalence of the bla_{TEM} gene requires further study, this gene might be widely disseminated in natural Korean environments. The genes encoding

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β-lactamases that hydrolyse clinically important third-generation cephalosporins such as cefotaxime are widespread in clinical and environmental Enterobacteriaceae isolates (Livermore 1995; Kim et al. 2005; Jacoby 2009; Jang et al. 2013). However, CTX-M-14- and DHA-1-encoding genes have not been reported previously in aeromonads. In addition, there is limited information about antibiotic resistance and its genetic background in *W. chitiniclastica*. To our knowledge, the present report is the first demonstrating that *A. hydrophila* and *W. chitiniclastica* harbour CTX-M-14-encoding genes and that *A. veronii* harbour a DHA-1 type AmpC-encoding gene.

In spite of the presence of a variety of genetic determinants, the present study revealed some discrepancies between antibiotic resistance phenotypes and genetic determinants among the isolates; for example, two isolates with *ampC* genes were susceptible to cefotaxime. Previous studies showed that induction of AmpC enzymes was dependent on regulatory genes (*ampR* and *ampD*) and relative *ampC* promoter strength (Barnaud et al. 1998; Jacoby 2009). Therefore, cefotaxime-susceptible isolates carrying the *ampC* gene might lack regulatory genes and/or might have a weaker *ampC* promoter sequence. Thus, although the discrepancy could be caused by the presence of a variety of other genetic determinants, it might due to insufficient production of proteins or mutations in the promoter sequences of genes for antibiotic resistance (Davies and Davies 2010; Wellington et al. 2013; Lazar et al. 2014).

This study is the first to profile bacterial pathogens and genetic determinants of antibiotic resistance from septicaemic Chinese soft-shelled turtles with ulcer disease. Many Gram-negative pathogens may collectively be associated with septicaemia in these animals. A multi-antibiotic resistance phenotype was frequently observed among the present isolates and all isolates harboured two or more genetic antibiotic resistance determinants. This finding might be due to the wide dissemination of antibiotic-resistant bacteria and resistance genes in aquatic environments around turtle farms.

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