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Original Article

Distribution of Genomic Species and Antimicrobial Susceptibility in Acinetobacters Isolated from Gangjin Bay, Korea

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A total of 90 *Acinetobacter* isolates from freshwater and seawater in Gangjin Bay of Korea was investigated for the distribution of genomic species, antimicrobial resistance patterns and clonal relatedness. By amplified ribosomal DNA restriction analysis, eighty-nine *Acinetobacter* isolates were classified into 11 *Acinetobacter* genomic species. *A. johnsonii* (n=23) was the most prevalent, followed by *A. baumannii* (n=13), *A. calcoaceticus* (n=13), *Acinetobacter* genomic species 11 (n=10), *A. phenon* 6/ct13TU (n=9), *A. junii* (n=5), *A. venetianus* (n=5), *Acinetobacter* genomic species 17 (n=4), 14BJ (n=3), *A. phenon* 10/1271 (n=2), *Acinetobacter* genomic species 3 (n=1), and ungrouped (n=1). The majority of *Acinetobacter* genomic species were isolated from the site A and B, and some known nosocomial pathogens in the clinical environment were observed among them. Of the 11 antimicrobial drugs tested, several *A. johnsonii* isolates exhibited high-frequency resistance to a wide variety of antimicrobial agents, including ampicillin-sulbactam, piperacillin, ceftazidime, cefotaxime, and sulfamethoxazole ($p < 0.001$). Some *Acinetobacter* genomic species were resistant to currently used antibiotics but all isolates were susceptible to imipenem, amikacin, and tetracycline. Based on the results of antimicrobial resistance pattern and phylogenetic analysis, 23 *A. johnsonii* isolates were classified into 19 pulsotypes. In conclusion, there was a significant difference in the distribution of *Acinetobacter* species between freshwater and seawater. Predominance of *A. johnsonii* strains was probably due to their ability to proliferate in the contaminated aquatic environment originated from local geographic features. Therefore, the waste effluent from animals and humans plays an important role in the distribution of *Acinetobacter* species in aquatic environment.

Key Words: *Acinetobacter johnsonii*, High-frequency resistance, Aquatic environment

INTRODUCTION

The genus *Acinetobacter* is aerobic Gram-negative non-fermentative cocobacilli that are ubiquitously distributed in environment, including soil, water, and sewage (1). In the past, these microorganisms were not highlighted in clinical setting, because they were considered to be a normal flora of skin or saprophytes in hospital environment. However, *Acinetobacter* species have emerged as important opportunistic pathogens in healthcare institutions due to the high prevalence in clinical specimens, occurrence of outbreaks by some *Acinetobacter* species, and its tendency to acquire multi-drug resistance (MDR) (2~4). In conjunction with its clinical importance, a systematic study for acinetobacters is

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needed to determine natural habitats, species distribution, and antimicrobial susceptibility in ecosystem.

Species identification of acinetobacters is essential to investigate the epidemiology of clinical isolates and to understand the natural occurrence of environmental isolates. Genus level of *Acinetobacter* can be presumptively identified by phenotypic markers, but the recent taxonomy of *Acinetobacter* species is based on the genotypic identification. Species identification of *Acinetobacter* is primarily based on DNA-DNA hybridization, but this method is laborious and is only available in a few reference laboratories (5). Many molecular methods, including amplified ribosomal DNA restriction analysis (ARDRA), amplified fragment length polymorphism, ribotyping, tRNA spacer fingerprinting, and sequence analysis of 16S~23S rRNA gene spacer region, have been developed for the identification of *Acinetobacter* genomic species (2, 4, 6, 7). Until now, 31 *Acinetobacter* genomic species were delineated, which were composed of 18 named and 13 unnamed species (8). Among them, *A. baumannii*, *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU, are the most frequently isolated from clinical specimens, whereas *A. calcoaceticus*, *A. johnsonii*, and *Acinetobacter* genomic species 11 are frequently found in soil and surface water. *Acinetobacter* isolates were heavily found in freshwater (> 10^4/100 ml) and raw sewage (10^6/100 ml) (9), but the distribution of *Acinetobacter* species in freshwater and seawater from shoreline was not fully determined by the current taxonomy.

In addition to their unique distribution in animate and inanimate environment, *Acinetobacter* species have become resistant to various classes of antimicrobial agents through mutation and selection, or by acquiring from other bacteria the genetic mechanism that encodes resistance. Most of clinical *Acinetobacter* isolates were MDR to clinically available antimicrobial agents, including penicillins/β-lactamase inhibitors, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems (10, 11). *Acinetobacter* isolates from natural environment were also resistant to naturally occurring or synthetic antimicrobial agents (12). Drug-resistant acinetobacters in aquatic environment would directly originate from animate environment or susceptible environmental bacteria could acquire resistance genes through horizontal gene transfer (13, 14). Aquatic environment has been known to be a reservoir for spreading of antimicrobial resistance by natural transformation or conjugation (15). This study was done to investigate the distribution of genomic species and antimicrobial susceptibility in *Acinetobacter* isolates from aquatic environment in Gangjin Bay, Korea. The clonal relatedness of the most predominant *A. johnsonii* isolates was also determined.

MATERIALS AND METHODS

Water sampling

The water was sampled from the Gangjin Bay, located in the southern extremity of Korea, for two times every spring season during 2004 and 2005. Four sampling sites were selected. Site A is influenced by freshwater inflowing from Tamjin River. Site B is influenced by a small quantity of seawater by the tidal currents. Sites C and D are influenced by the ocean currents, but site D is more seaside than site C. The majority of the inhabitants living in surrounding area of the Gangjin Bay raise livestock such as cows, pigs and poultry, and the waste effluent flows into site A.

The water samples were collected in a sterile bottle at a high tide from the shoreline of the Bay. To culture bacteria, water samples were filtered through a 0.7 μm nylon net (Falcon Cell Strainer, BD Biosciences, San Jose, CA, USA) to remove any impurities and then filtered through a membrane filter with a pore size of 0.45 μm (Millipore Co., Milford, MA, USA). The filtered membranes were then laid onto an enrichment medium for *Acinetobacter* species (0.15% KH_2PO_4, 1.65% $Na_2HPO_4 \cdot 2H_2O$, 0.00005% $FeSO_4 \cdot 7H_2O$, 0.02% $MgSO_4 \cdot 7H_2O$, 0.01% $CaCl_2$ and 0.2% $C_2H_3O_2Na$, pH, 7.5) and incubated at 30℃ for 48 h (9, 16). A well-isolated colony was subcultured onto a MacConkey agar plate (Difco Laboratories, Detroit, MI, USA) using a toothpick.

Physicochemical test of sampling water

Physicochemical parameters from the four sampling

sites were directly measured in the field. The ambient water temperature (Digital thermometer, IBRD YSI Model 58, Springfield, NJ, USA), pH (Orion EA 940, Beverly, MA, USA), salinity (Salinity meter, IBRD YSI Model 33) and the nutritional salts for nitrogen-nitrate (NO_3-N) and phosphate phosphorus (PO_4-P) were analyzed.

Identification of *Acinetobacter* genomic species

Acinetobacter genomic species was identified by previously described ARDRA method (2). After amplification of 16S ribosomal DNA region by PCR, 10 μl of each PCR product was digested for 2 h at 37℃ in 20 μl volumes of commercially supplied incubation buffer containing 5 U of respective restriction enzyme *Alu*I, *Cfo*I, *Mbo*I, *Msp*I, and *Rsa*I (Roche Diagnostics GmbH, Mannheim, Germany). Restriction fragments were separated by agarose gel electrophoresis in 0.5 × TBE buffer and visualized after being stained with ethidium bromide.

Growth of *Acinetobacter* species by different salt concentration

The growth rates of five *Acinetobacter* species were investigated under various salinity conditions. One milliliter of mid-logarithmic culture adjusted to optical density (OD) 1 at 600 nm was added to the 250 ml triangle flask containing 100 ml enrichment medium with 1%, 2% and 3% (w/v) of sodium chloride, respectively, and then shaken vigorously for 48 h at 30℃. The OD value of each culture was measured every 6 h.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed using the agar plate method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (17). A fixed concentration of antimicrobial agents was incorporated into the Mueller Hinton agar (Difco Laboratories). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. The antimicrobial agents for MIC test were as follows: ampicillin-sulbactam (Sigma Chemical Co., St. Louis, MO, USA), piperacillin (ICN Biomedicals, Irvine, Ca, USA), ceftazidime (Sigma Chemical Co.), cefotaxime (DUCHEFA, Haarlem, Netherland), imipenem (Sigma Chemical Co.), colistin (Sigma Chemical Co.), amikacin (ICN Biomedicals), gentamicin (DUCHEFA), tetracycline (Sigma Chemical Co.), ciprofloxacin (Fluka, Buchs, Switzerland), sulfamethoxazole (Sigma Chemical Co.).

Pulsed-field gel electrophoresis (PFGE)

The PFGE plugs were prepared according to a modified version of the Gautom method (18) and the genomic DNA was digested with *Apa*I (Roche Diagnostics GmbH) for 20 h and separated on a 1% agarose gel using a contour-clamped homogeneous apparatus (CHEF DRIII systems; Bio-Rad Co, Hercules, CA, USA). The agarose plugs were digested with 50 U of *Apa*I at 37℃ for 24 h. A lambda ladder comprising 48.5-kb concatemers (Roche Diagnostics GmbH) was used as size standards. The agarose gels were run in 0.5 × TBE buffer at 14℃ at 6 V/cm for 19 h with a pulse time 5 to 20 s with a linear ramp. The gel images were stored electronically as TIFF files and analyzed using GelCompar II computer program (Applied Maths Co., Sine-Martens-Latem, Belgium) for band analysis with the Dice coefficient used to compute the similarity matrix and then transformed with the arithmetic average (UPGMA).

Statistical analysis

The statistical analysis was done with SPSS version 11.5 for Microsoft windows. The statistical significance of difference between groups was analyzed by Student's t-test and χ^2 test.

RESULTS

Physiochemical characterization of survey water

The physicochemical parameters of sampling waters were summarized in Table 1. There was no significant difference in water temperature among the sampling sites. The pH and salinity of sites C and D showed a typical seawater, whereas site A was influenced by freshwater and site B was a crossing point between freshwater and seawater. The total nitrogen and phosphate phosphorus were significantly higher

Table 1. Physicochemical characteristics of sampled water at survey sites

Item	Year	Survey site			
		Site A	Site B	Site C	Site D
Temperature (℃)	2004	16.6	15.9	15.3	15.1
	2005	16.7	16.1	16.4	15.5
pH	2004	7.3	7.9	8.1	8.1
	2005	7.4	7.9	8.1	7.9
Salinity (‰)	2004	8.9	24.8	31.8	32.3
	2005	14.1	21.8	27.5	28.4
Total nitrogen (mg/L)	2004	0.872	0.356	0.164	0.142
	2005	1.059	0.352	0.178	0.171
Phosphate phosphorus (mg/L)	2004	0.064	0.037	0.019	0.013
	2005	0.058	0.028	0.014	0.017

at site A than the other sampling sites due to the waste effluent from farms and villages.

Table 2. Distribution of *Acinetobacter* genomic species in Gangjin Bay in Korea

Genomic species	No. of isolates from sites				
	A	B	C	D	Total
A. johnsonii	14	9	0	0	23
A. calcoaceticus	8	4	0	1	13
A. baumannii	7	5	0	1	13
11	2	8	0	0	10
A. phenon 6/ct13TU	8	1	0	0	9
A. junii	6	0	0	0	6
A. venetianus	0	2	2	1	5
17	0	0	3	1	4
14BJ	1	0	2	0	3
A. phenon 10/1271	2	0	0	0	2
3	0	1	0	0	1
Ungrouped	0	1	0	0	1
Total	48	31	7	4	90

Distribution of *Acinetobacter* genomic species

A total of 90 *Acinetobacter* isolates were obtained from four sampling sites. Forty-eight (53.3%) isolates were obtained from freshwater, 31 (34.4%) isolates were from crossing point between the freshwater and the seawater and 11 (12.2%) isolates were found in seawater (Table 2). Of the 90 *Acinetobacter* isolates, 89 isolates were classified into 11 *Acinetobacter* genomic species. Genomic species of one *Acinetobacter* isolate from site B was not determined by ARDRA. Overall, *A. johnsonii* (n=23) was the most prevalent, followed by *A. baumannii* (n=13), *A. calcoaceticus* (n=13), and *A. phenon* 6ct/13TU (n=9), which were all predominant species at sites A and B. There was a great difference in the distribution of *Acinetobacter* species between the sampling sites. *A. junii* and *Acinetobacter* genomic species 10 were only found in freshwater, whereas *Acinetobacter* genomic species 17 were isolated from seawater.

To determine whether the unique distribution of *Acinetobacter* species in freshwater and seawater was influenced by the salinity of the sampled water, five *Acinetobacter* species were cultured in the medium supplemented with 1%, 2% and 3% salinity, and the optical density of the bacterial growth was measured using a spectrophotometer. Three predominant species at both sites A and B, *A. calcoaceticus*, *A. baumannii*, and *A. johnsonii*, were selected and *A. venetianus* and *Acinetobacter* genomic species 17, which were predominant at sites C and D, were selected. The growth of all *Acinetobacter* species tested slightly decreased with the increasing of salinity, but there was no significant difference in the growth rates of each *Acinetobacter* species under the different salinities. However, three predominant *Acinetobacter* species at sites A and B displayed higher growth rate than two selected *Acinetobacter* species at sites C and D under 1% and 2% salinity condition.

Antimicrobial susceptibility of aquatic *Acinetobacter* isolates

The MICs of 11 antimicrobial agents against all 90 *Acinetobacter* isolates were determined by agar dilution method. Antimicrobial resistance patterns, MICs, and resistance percentages are presented in Table 3. Four (4.4%) *A. johnsonii* of all *Acinetobacter* isolates were only

Table 3. MICs and the percentages of resistance (%R) of 90 *Acinetobacter* isolates from Gangjin Bay of Korea

Acinetobacter genomic species (n=90)	MIC_{90} and %R of [a]										
	AMS[b]	PIP	CAZ	CTX	IP	CS	GM	AMK	CIP	TET	SMX
A. johnsonii (n=23)	>128 / 4.4	≥512 / 5.6	128 / 10.0	128 / 4.4	<1 / 0	<1 / 0	<4 / 1.1	2 / 0	0.5 / 2.2	4 / 0	≥1,024 / 7.8
A. calcoaceticus (n=13)	4 / 0	≥512 / 2.2	4 / 1.1	16 / 0	<1 / 0	<1 / 0	<4 / 0	4 / 0	<0.25 / 0	2 / 0	32 / 0
A. baumannii (n=13)	4 / 0	≥512 / 2.2	8 / 0	16 / 0	<1 / 0	4 / 0	<4 / 0	8 / 0	<0.25 / 0	4 / 0	16 / 0
11 (n=10)	2 / 0	64 / 1.1	8 / 1.1	16 / 0	<1 / 0	8 / 5.6	<4 / 0	4 / 0	8 / 4.4	4 / 0	16 / 0
A. phenon 6ct:13TU (n=9)	4 / 0	≥512 / 3.3	8 / 0	16 / 0	<1 / 0	<1 / 0	<4 / 0	4 / 0	<0.25 / 0	2 / 0	<8 / 0
A. junii (n=6)	<0.5 / 0	32 / 1.1	4 / 0	8 / 0	4 / 0	8 / 3.3	<4 / 0	4 / 0	0.5 / 0	4 / 0	64 / 1.1
A. venetianus (n=5)	<0.5 / 0	16 / 0	4 / 0	8 / 0	<1 / 0	4 / 0	<4 / 0	8 / 0	<0.25 / 0	2 / 0	<8 / 0
17 (n=4)	2 / 0	8 / 0	<2 / 0	<2 / 0	<1 / 0	4 / 0	<4 / 0	2 / 0	<0.25 / 0	4 / 0	16 / 0
14BJ (n=3)	2 / 0	32 / 0	8 / 0	16 / 0	<1 / 0	4 / 0	<4 / 0	4 / 0	8 / 1.1	2 / 0	<8 / 0
A. phenon 10/1271 (n=2)	4 / 0	8 / 0	<2 / 0	4 / 0	<1 / 0	<1 / 0	<4 / 0	4 / 0	<0.25 / 0	2 / 0	<8 / 0
3 (n=1)	1 / 0	32 / 0	4 / 0	16 / 0	<1 / 0	<1 / 0	<4 / 0	2 / 0	<0.25 / 0	2 / 0	<8 / 0
Ungrouped (n=1)	4 / 0	<4 / 0	<2 / 0	4 / 0	<1 / 0	8 / 1.1	<4 / 0	2 / 0	<0.25 / 0	4 / 0	<8 / 0

[a] AMS, ampicillin-sulbactam (MIC range, 0.5 to 128 μg/ml); PIP, piperacillin (4 to 512 μg/ml); CAZ, ceftazidime (2 to 128 μg/ml); CTX; cefotaxime (2 to 256 μg/ml); CS; colistin (1 to 32 μg/ml); AMK, amikacin (1 to 64 μg/ml); GM, gentamicin (4 to 256 μg/ml); TET, tetracycline (1 to 16 μg/ml); CIP, ciprofloxacin (0.25 to 32 μg/ml); SMX, sulfamethoxazole (8 to 1,024 μg/ml).
[b] MIC values to sulbactam are omitted

resistant to ampicillin-sulbactam (the MICs for 90% of the strains tested was ≥128/64 μg/ml). The *Acinetobacter* genospecies for piperacillin (PIP) with MIC_{90} (≥512 μg/ml) were *A. johnsonii* (5.6%), *A. calcoaceticus* (2.2%), *A. buamannii* (2.2%), and *A. phenon* 6ct/13TU (3.3%). Five (5.6%) *A. johnsonii* among the 6 *Acinetobacter* genomic species resistant to piperacillin showed high resistance rate. Three *Acinetobacter* genospecies (9 [10.0%] *A. johnsonii*, 1 [1.1%] *A. calcoaceticus*, and 1 [1.1%] *Acinetobacter* genomic species 11) were resistant to ceftazidime and four (4.4%) *A. johnsonii* were also resistant to cefotaxime with MIC_{90} (128 μg/ml) in agreement with ampicillin-sulbactam and ceftazidime MICs tested. Five (5.6%) *Acinetobacter* genomic species 11 were resistant to colistin (breakpoint, ≥4 μg/ml) with MIC_{90} (8 μg/ml), followed by 3 (3.3%) *A. junii*, and 1 (1.1%) Ungrouped. Only one (1.1%) *A. johnsonii* among all the isolates was resistant to gentamicin (≥256 μg/ml). Four (4.4%) *Acinetobacter* genomic species 11, 2 (2.2%) *A. johnsonii*, and 1 (1.1%) 14BJ were resistant to ciprofloxacin (breakpoint, ≥4 μg/ml). Especially, the MICs (16 μg/ml) against two *A. johnsonii* isolates were twofold higher than those with others resistant strains. Seven (7.8%) *A. johnsonii* and one (1.1%) *A. junii* exhibited a high level of resistance to sulfamethoxazole (MIC_{90}, ≥1,024 μg/ml).

Epidemiological relationship of aquatic *A. johnsonii* isolates

A total of 23 *A. johnsonii* isolates were analyzed to determine their clonal relatedness. Thirteen *A. johnsonii* isolates from site A were classified into 11 pulsotypes and 10 isolates from site B were classified into 8 pulsotypes at a similarity value of 0.85 (Fig. 1). Two *A. johnsonii* isolates of site B showed identical PFGE pattern, but they showed different antimicrobial resistance patterns to several beta-lactam antibiotics.

DISCUSSION

In the present study, 90 *Acinetobacter* strains collected from Gangjin Bay of Korea were investigated for genotypic identity and antibiotic susceptibility under the ecological

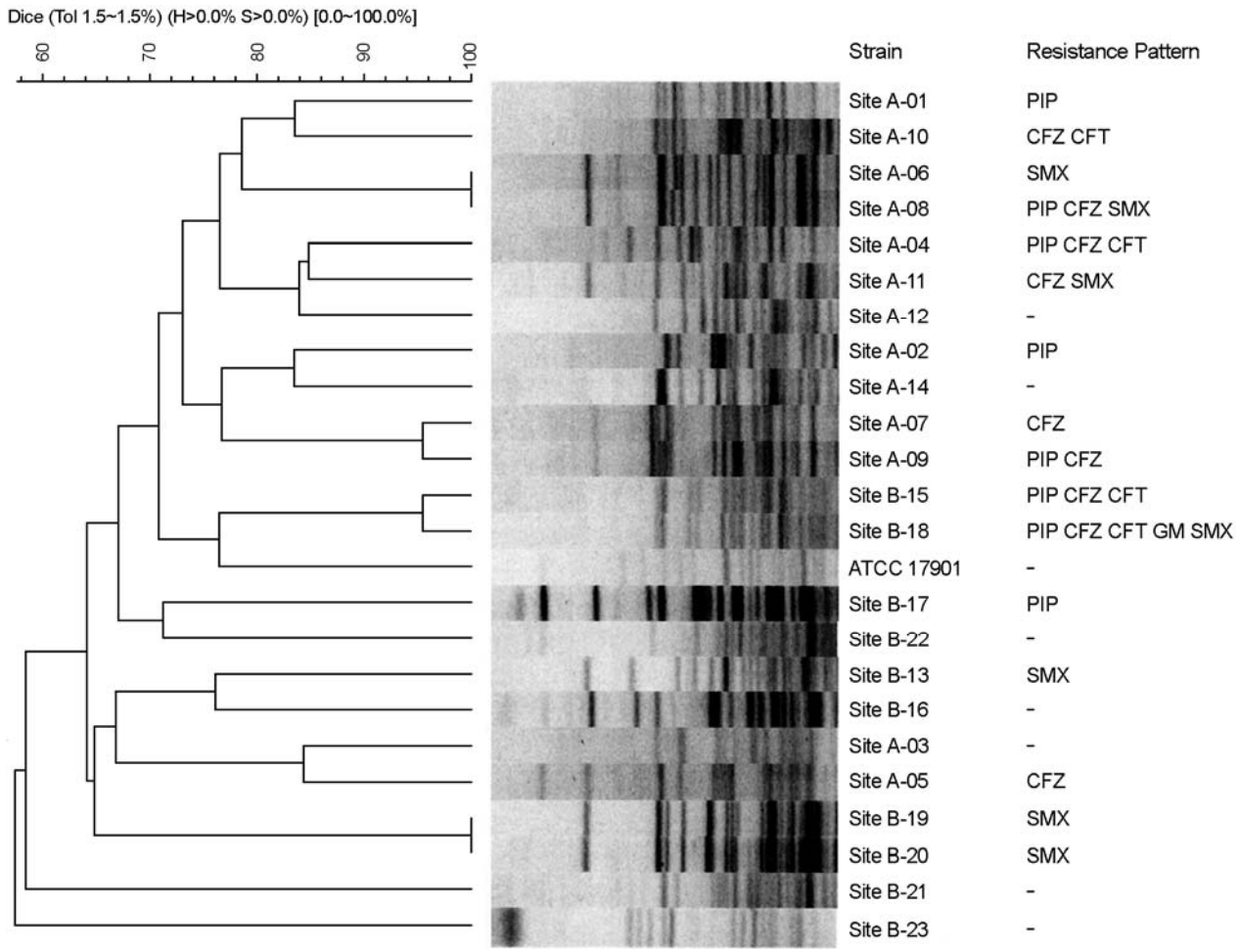

Figure 1. Phylogenetic analysis of 23 *A. johnsonii* isolates from the Gangjin Bay of Korea. CHEF electrophoresis of *Apa*I-digested genomic DNAs. The dendrogram is based on cluster analysis by the unweighted-pair group method with average linkages.

condition of sampling sites. Epidemiological typing of predominant *A. johnsonii* isolates was also analyzed by PFGE. The results of the physiochemical characterization are summarized in Table 1. There was no difference in water temperature. Seveno *et al* (19) described that bacteria can proliferate more lively in the environmental conditions containing organic sources, solid surface, sediments, and nutrition (phosphate and nitrates) necessary for division of bacteria in aquatic environment. In case of site A, bacterial growth condition seems to be better than other sampling sites with regards to the salinity and total nitrogen and phosphate phosphorus concentrations. Accordingly, site A could display dominant microorganisms originated from freshwater or excreta of humans and animals.

The growth rates of five selected *Acinetobacter* species were not significantly different under 3% salinity. These results suggest that the unique distribution of *Acinetobacter* species between freshwater and seawater could have been influenced by other factors than the salinity. A high prevalence of various *Acinetobacter* species at site A is possibly due to the waste effluent from farms and villages.

The distribution of *Acinetobacter* species in the inanimate environment has been studied and demonstrated that there were some differences in the distribution of *Acinetobacter* species according to the geographical regions. *A. calcoaceticus*, *A. johnsonii*, *A. haemolyticus*, and *Acinetobacter* genomic species 11 were found most frequently in soil and surface water in Germany (20). *Acinetobacter*

isolates were found in 22 of 60 soil samples in Hong Kong and the most frequent species were *Acinetobacter* genomic species 3 and *A. baumannii* (21). Berlau *et al* (22) demonstrated that *Acinetobacter* isolates were detected in 30 of 177 vegetables from the United Kingdom and that *A. baumannii* and *Acinetobacter* genomic species 11 were the most predominant species. *A. baumannii* and *Acinetobacter* genomic species 13TU are important hospital-acquired pathogens and are occasionally an etiologic agent of animal infection (21). *A. phenon* 6/ct13TU strains have been reported from a University hospital of Korea (23) but they do not appear to be a typical environmental species. In this study, *A. johnsonii*, *A. calcoaceticus*, *A. baumannii*, *Acinetobacter* genomic species 11, and *A. phenon* 6/ct13TU were found to be the predominant species. Thus, those isolates found at site A and B could have originated from the waste effluent from villages or soil. *A. venetianus* and *Acinetobacter* genomic species 17 found at site C and D would be aboriginal microorganisms that survive in seawater rather than freshwater.

The antimicrobial resistance patterns of 90 *Acinetobacter* isolates were determined. Overall, the *Acinetobacter* isolates from Gangjin Bay were found to exhibit a moderate level to high frequency of resistance to ampicillin-sulbactam (4.4%), piperacillin (15.6%), ceftazidime (12.2%), colistin (10.0%), ciprofloxacin (8.9%), sulfamethoxazole (8.9%), cefotaxime (7.8%), and gentamicin (1.1%) but all isolates were susceptible to imipenem, amikacin, and tetracycline. Several *A. johnsonii* isolates exhibited high-frequency resistance to a wide variety of antimicrobial agents, including ampicillin-sulbactam, piperacillin, ceftazidime, cefotaxime, and sulfamethoxazole ($p < 0.001$). *A. johnsonii*, known as a nosocomial pathogen, considered a commensal on human skin, and that has been rarely reported from the hospitalized patients as well as clinical setting in Korea (24). In addition, antimicrobial resistance patterns against those isolates were not common. As a result, *A. johnsonii* isolates showing MDR patterns different from other *Acinetobacter* genomic species were probably autochthonous strains living in the survey area.

Interestingly, *Acinetobacter* genomic species 11 showed a high frequency of resistance to colistin and ciprofloxacin as compared to other *Acinetobacter* genomic species. Recently, the isolation frequency of MDR *A. baumannii* strains including carbapenem-resistant have been increased in the clinical setting (25, 26). *A. baumannii* isolates in this study were susceptible to those antibiotics, and had low frequency of resistance, whereas resistance to colistin focused on the *Acinetobacter* genomic species 11 and *A. junii* than *A. johnsonii* isolates resistant to antimicrobial agents. It is suggested that colistin-resistant *Acinetobacter* isolates were first found in the aquatic environment of Gangjin Bay, as opposed to that have not yet been reported in the clinical isolates.

Sulfamethoxazole-resistant *Acinetobacter* isolates in this study demonstrated high-level resistance equal to that shown by enteric gram-negative bacteria or nosocomial organisms isolated from hospital environment. In a previous preliminary study, eight sulfamethoxazole-resistant *Acinetobacter* isolates from the survey area were examined for *sul1* and *sul2* gene according to the method described by Park *et al* (27). As a result, the *sul2* gene was only detected in five *A. johnsonii* isolates but the remaining isolates were negative. In addition, class 1 integron including integrase gene as well as *sul1* gene also had not been found (data not shown). Studies examining trimethoprim/sulfamethoxazole-resistant *Escherichia coli* and *Stenotrophomonas maltophilia* isolates recently reported that some of those isolates possessed *sul2* gene carrying large plasmids, whereas others carried *sul1* gene on the class 1 integrons (28, 29). Thus, it was speculated that would be present resistance determinants encoding sulfamethoxazole in the *sul2*-positive *A. johnsonii* isolates.

Acinetobacter isolates from aquatic environment were more susceptible to antimicrobial agents as compared to the MDR gram-negative bacteria in clinical isolates from humans and animals and some *Acinetobacter* species are intrinsically resistance to β-lactams, some cephalosporins, and chloramphenicol (30), which are responsible for the prevalence of resistance to these antimicrobial agents in this study. Moreover, aquatic acinetobacters can acquire resistance determinants by the horizontal gene transfer (31). Resistance to folate pathway inhibitors such as trimethoprim

and sulfamethoxazole was infrequently found in the bacteria from aquatic environment. Since resistance determinants of antifolate agents were usually located in the plasmids of gram-negative bacteria, trimethoprim resistance conferring dihydrofolate reductase genes and various types of *sul* genes could be transferred to *Acinetobacter* species in aquatic environment (32). Since a high level of resistance to fluoroquinolones is mediated by chromosomal mutation of DNA gyrases (33), resistance to fluoroquinolones has been known as the critical indicator to reveal inflow of the waste products from animate environment (34). As a result, some of *Acinetobacter* genomic species resistant to various classes of antimicrobial agents in this study would be supposed to originate from humans or animals, whereas other *Acinetobacter* isolates susceptible to β-lactam, β-lactamase inhibitor, cephalosporins and aminoglycosides, and folate pathway inhibitors were probably autochthonous strains living in the Gangjin Bay. Based on the results of antimicrobial susceptibility and phylogentic analysis, 23 *A. johnsonii* isolates were classified into 19 plusotypes. The genetic heterogeneity of *A. johnsonii* isolates is possibly due to the influx of new clones from the waste effluent and/or the animate environment. In conclusion, we demonstrated the species distribution and antimicrobial susceptibility patterns of *Acinetobacter* isolates from aquatic environment of Gangjin Bay. There was a significant difference in the distribution of *Acinetobacter* species between freshwater and seawater. Interestingly, *A. baumannii* was found to be one of the predominant species in aquatic environment in Korea, but *A. baumannii* isolates did not show MDR phenotype, probably suggesting the autochthonous strains in aquatic environment. Moreover, the predominance of *A. johnsonii* isolates was probably due to their ability to proliferate in the aboriginal aquatic environment as well as fecal contamination of animal and human originated from local geographic features. Consequently, the waste effluent from animals and humans plays an important role in the distribution of *Acinetobacter* species in aquatic environment. Further studies for *Acinetobacter* species in ecosystem are mandatory to increase our knowledge of the antimicrobial resistance mechanisms and epidemiological relationship of *Acinetobacter* species in association with clinical isolates.

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