Understanding molecular features of carbapenem-resistant *Acinetobacter baumannii* causing invasive infection through comparative genomics and their clinical implications

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Directed by Professor Jun Yong Choi

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I am much honoured to present this manuscript entitled "**Understanding molecular features of carbapenem-resistant *Acinetobacter baumannii* causing invasive infection through comparative genomics and their clinical implications**" to distinguished scholars as you.

Considering the threat of increasing incidence of carbapenem-resistant *Acinetobacter baumannii* **(**CRAB) infection, prediction of the future effects of these isolates is a priority. Whole genome sequencing (WGS) analysis provides unprecedented information about this illusive organism, especially for the characterisation of nosocomial outbreaks. However, whether the molecular characteristics of CRAB causing invasive infection have changed over time is questionable and its clinical significance is unclear. We, therefore, attempted whole genome-association study through sequencing of all available isolates from patients with CRAB bacteraemia to characterise the genomic changes over the study period from the epidemiological perspective and investigated the clinical significance of the genomic characteristics of CRAB.

I thank all the nursing and laboratory staff and physicians who supported this project. Finally, I give credit to all the patients included in the study. I would like to thank professor Jun Yong Choi for his dedication to this manuscript.

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ABSTRACT

Understanding molecular features of carbapenem-resistant *Acinetobacter baumannii* causing invasive infection through comparative genomics and their clinical implications

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Acinetobacter baumannii is a highly potent nosocomial pathogen, associated with increased in-hospital mortality. Here, we investigated the changes in the molecular characteristics of carbapenem-resistant *A. baumannii* (CRAB) isolated from the blood samples of patients admitted to a tertiary hospital in South Korea from January 2009 to July 2015. Whole genome sequencing (WGS) using the Illumina MiSeq platform and multi-locus sequence typing (MLST) were performed for 98 CRAB clinical isolates. Antimicrobial resistance genes were identified by querying contig sequences against ResFinder database. The distribution of virulence factors was obtained using Large Scale blast score ratio (LS-BSR). Epidemiologic data were collected from hospital database. MLST using Oxford scheme revealed 10 sequence types of CRAB, of which ST191 was the dominant type (n = 59). Although $bla_{\text{OXA-23}}$ was shared by most analysed strains, the composition of antimicrobial resistance determinants was different among the sequence types. ST447 and ST451/ST1809 with a few resistance genes were isolated during the later years of the study period. Number of virulence genes increased, while that of ST191 did not changed significantly during the investigation period. MLST types, composition of antimicrobial resistance genes, and virulence genes had no association with clinical outcomes of CRAB bacteraemia. In conclusion, the active change in or accumulation of antimicrobial resistance determinants and virulence genes in CRAB was not observed during the research period. The molecular characteristics of CRAB had no association with clinical outcomes of

CRAB bacteraemia.

Keywords: carbapenem-resistant *A. baumannii*, whole genome sequencing, antimicrobial determinant, virulence genes

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I. INTRODUCTION

Acinetobacter baumannii is an aerobic gram-negative coccobacillus known for relatively few virulence factors as compared to other gram-negative pathogens [1]. Its ability to acquire various antimicrobial resistance genes deems it as a highly successful nosocomial pathogen, which is associated with increased in-hospital mortality [2]. In particular, the prevalence of carbapenem-resistant *A. baumannii* (CRAB) isolates is on the rise in Asian countries, including South Korea. The specific clone, international clone 2 (IC II), is a major clonal group among Korean CRAB isolates [3]. This organism is associated with nosocomial outbreaks and multidrug resistance in association with $bla_{\text{OXA-23}}$-like genes [4]. In a recent study, 75% of *A. baumannii* clinical isolates were shown to belong to IC II in Korea [5], and IC II was recognised as the second most common cause of bacteraemia from 2012 to 2013 [6].

Considering the threat of increasing incidence of CRAB infection, prediction of the future effects of these isolates is a priority. Whole genome sequencing (WGS) analysis provides unprecedented information about this illusive organism, especially for the characterisation of nosocomial outbreaks [7,8]. WGS provides in-depth epidemiologic knowledge by dividing organisms into sequence types (STs) and suggesting the emergence of new epidemic clones. WGS also helps to understand the various

mechanisms underlying the acquisition of antimicrobial resistance genes. Antimicrobial resistance genes were found in alien islands of accessory genomes and were often accompanied with integrases, transposases, or insertion sequences, suggestive of their possible acquisition by horizontal gene transfer from other *Acinetobacter* spp. or bacteria that colonise the same environment [9,10]. Furthermore, although little is known regarding the virulence factors of this bacterium as compared to the current knowledge about antimicrobial resistance mechanism [11], the rapid increase in the number of sequenced and annotated virulence genomes has enabled comparative genomic analyses to elucidate clues concerning potential virulence factors.

Whether the molecular characteristics of CRAB causing invasive infection have changed over time is questionable and its clinical significance is unclear. A few studies have investigated the genomic changes in CRAB isolated from blood in association with clinical characteristics of hosts in a real hospital setting. We, therefore, attempted whole genome-association study through sequencing of all available isolates from patients with CRAB bacteraemia to characterise the genomic changes over the study period from the epidemiological perspective and investigated the clinical significance of the genomic characteristics of CRAB. These investigations on the genetic characteristics, including antimicrobial resistance determinants and virulence factors of CRAB blood isolates, over time can provide insight into how the organism evolves and may suggest clinico-epidemiological implications of the genomic characteristics of this organism.

II. MATERIALS AND METHODS

1. Collection of bacterial isolates and patient information

Blood isolates from patients with CRAB bacteraemia were collected between January 2009 and July 2015 at intensive care units (ICUs) of Severance Hospital in Seoul, Republic of Korea. CRAB bacteraemia was defined as one or more positive blood cultures for carbapenem-resistant *A. baumannii* and the presence of clinical features compatible with systemic inflammatory response syndrome. Preliminary species identification and antimicrobial susceptibility tests were carried out with VITEK II system (bioMérieux, Marcy l'Etoile, France). Disc-diffusion testing using antimicrobial

discs (Becton Dickinson, Sparks, MD, USA) on cationic-adjusted Mueller–Hinton (MH) agar (Difco Laboratories, Detroit, MI, USA) was subsequently performed as per CLSI guidelines [12]. The minimum inhibitory concentrations (MICs) of imipenem and meropenem were determined with the agar dilution method using MH agar following CLSI guidelines [12]. Additionally, modified Hodge tests and double disk synergy tests were carried out to screen carbapenemase and metallo-β-lactamase activity, respectively.

We collected clinical data, including patient age and sex, length of ICU stay, length of hospital stay, pre-existing chronic comorbidities (diabetes, chronic heart failure, chronic liver disease, chronic renal disease, chronic pulmonary disease), sequential organ failure assessment score, episodes of hospital admission, previous invasive procedure (central line insertion, intubation, continuous renal replacement therapy, surgery under general anaesthesia), and length and kinds of antibiotics treatment. Origin of bacteraemia was determined upon identification of precedent CRAB isolation or evidence of infection before the event of bacteraemia.

2. WGS, annotation, prediction of antimicrobial resistance determinants, and identification of virulence factor genes

The isolates were cultured in Luria Bertani (LB) broth and genomic DNA was extracted with PureHelix™ Genomic DNA Prep Kit (cat. no. GCTN100, Nanohelix, Daejeon, Republic of Korea). WGS of 98 *A. baumannii* clinical isolates was carried out using the Illumina MiSeq platform at ChunLab (Seoul, Republic of Korea). *De novo* assembly of the raw sequencing reads was performed using CLC Genomics Workbench v11.0.1 (https://www.qiagenbioinformatics.com/). The assembled genome sequences were annotated using the NCBI's Prokaryotic Genome Annotation Pipeline (PGAP) v4.5[13]. Phylogenetic tree was constructed using the FastTree2 [14] and visualised using the iTOL server [15].

To identify antimicrobial resistance genes, the contig sequences were queried against ResFinder database (https://cge.cbs.dtu.dk/services/data.php; downloaded on June 4, 2018) using the Find Resistance v1.01 from the Microbial Genomics Module under CLC Genomics Workbench.

Comprehensive list of virulence factors from *A. baumannii* was compiled as per the previous studies[16-19]. The list of 182 virulence genes is shown in Supplementary Table S2. The distribution of virulence genes across all genomes was obtained using Large Scale blast score ratio (LS-BSR) [20]. The

value of 0.7 was arbitrarily used as a cutoff to evaluate the existence of each virulence gene.

3. Multi-locus sequence typing (MLST) and detection of single nucleotide polymorphism (SNP)

Allelic profiles or ST of each isolate were determined by submitting contig sequences to PubMLST site (https://pubmlst.org/abaumannii/) according to Institute Pasteur scheme (MLST-IP) and Oxford Database (MLST-OD).

NPs were identified by mapping raw sequencing reads to the complete chromosome sequence of *A. baumannii* strain KBN10P02143 (GenBank CP013924.1), a multidrug strain isolated from Korea in 2012, using Snippy (https://github.com/tseemann/snippy).

4. Identification of insertion sequences and transposons

The $bla_{\text{OXA-23}}$ is a major determinant of nosocomial outbreak of IC II CRAB. The expression of this gene is presumably regulated by several insertion sequences, including IS*Aba1*, which is thought to play the most crucial role in gene expression [21]. ISMapper [22] was used for the identification of IS*Aba1* insertion sites on the reference genome of the strain KBN10P02143. Insertion sites of IS*Aba1* relative to the chromosome of KBN10P02143 are shown in Supplementary Table S3. The presence of $bla_{\text{OXA-23}}$-carrying transposons, including Tn*2006*, Tn*2007*, Tn*2008*, and Tn*2009*, were screened using Primersearch from EMBOSS package (http://emboss.sourceforge.net/), with primer sets suggested by Chen *et al* [23].

5. Statistical analysis

Baseline characteristics were compared using Mann–Whitney U test or independent samples *t*-test for continuous variables, and χ^2 test or Fisher's exact test for categorical variables. Continuous variables were expressed as means or medians (interquartile ranges), while categorical variables were expressed as numbers with percentages for the description of baseline characteristics. Statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

6. Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB) of Yonsei University Health System Clinical Trial Center (4-2017-0730). Since the study had minimal health risk and the study subjects were anonymized, the Institutional Review Board waived the requirement for written informed consent from the patients.

7. Data availability

The genome sequences were submitted to GenBank under BioProject no. PRJNA448358 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA448358). The complete list of strain names and accession numbers are shown in Supplementary Table S1.

III. RESULTS

1. Overview of *A. baumannii* isolates

A total of 109 CRAB blood isolates were used in this study. Of these, three samples with possibility of contamination and eight samples with insufficient clinical data were excluded. The remaining 98 samples were consequently involved in the analysis. The isolates exhibited a high rate of resistance for almost all the antibiotics tested as follows: 92.7% to gentamycin (89/96), 88.8% to amikacin (87/98), 100% to imipenem (98/98), 98.8% to ciprofloxacin (84/85), 97.8% to levofloxacin (96/98), 98.9% to piperacillin-tazobactam (92/93), 98.9% to ceftazidime (96/97), 96.9% to cefepime (95/98), 4.3% to colistin (88/92), and 11.1% to tetracycline (1/9).

2. MLST analysis depending on isolation years

MLST-IP (Pasteur) scheme identified 93 strains (94.9%) being classified into ST2, indicating that most of the strains belongs to the International Clone II (IC II). MLST using Oxford scheme [24,25] was used to assign 10 STs (ST858, ST369, ST208/ST1806, ST784, ST451/ST1809, ST357, ST368, ST447,

ST552, and ST191). ST191 was the most abundant one (59 strains). Strains ABAY12003 and ABAY13017, single variant of ST191 at *gyrB* and *rpoD*, respectively, were not assigned any STs. As a result, we found that 91 strains (92.9%), all belonging to IC II, had two incidences of *gdhB* alleles, 3 and 189, which made distinctions between ST208 and ST1806 and ST451 and ST1809 ambiguity. All ST191 strains had two *gdhB* alleles, but only ABAY15007 strain harbouring *gpi*-94 *gpi*-107 was equivocally assigned to ST191 and ST784.

ST191 was evenly isolated throughout the research period. ST368 (n = 3), ST552 (n = 1), ST369 (n = 3), ST357 (n = 7), and ST208/ST1806 (n = 5) were mostly isolated from 2009 to 2012, while ST447 (n = 4), ST451/ST1809 (n = 11), ST858 (n = 1), ST784 (n = 1), and ST191/ST784 (n = 1) were mostly reported from 2013 to 2015 (Fig. 1, Table 1).

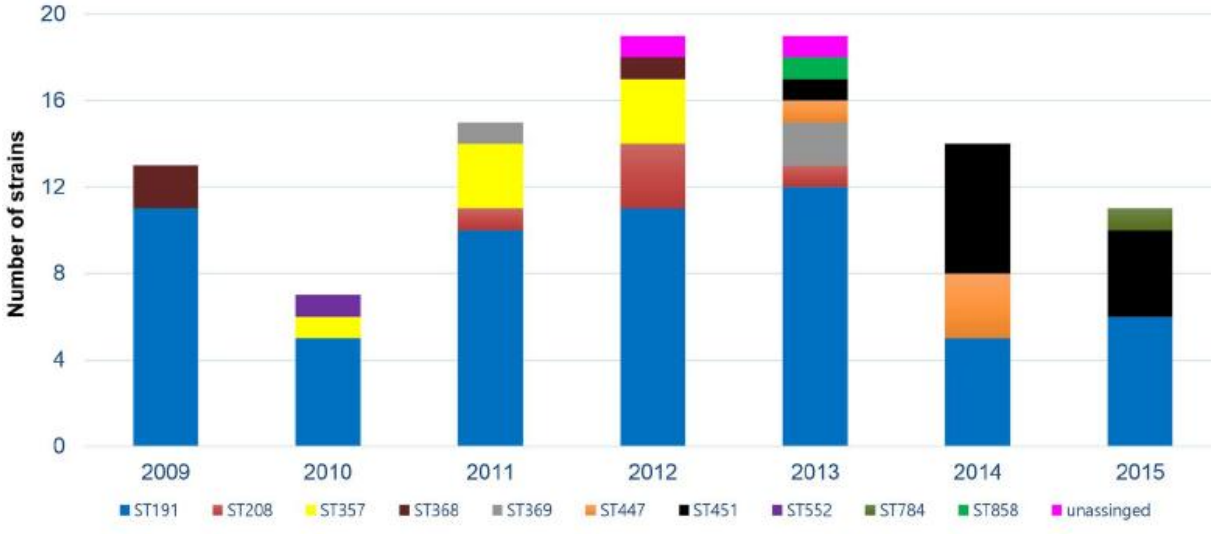

Figure 1. Distribution of carbapenem resistance *A. baumannii* isolates according to sequence types and year of isolation. The isolates were typed by multi-locus sequence typing (MLST) using the Oxford scheme and colored individually according to sequence type. The bar graph represents the individual and cumulative numbers of strains isolated each year.

Table 1. Sequence types of carbapenem resistant *A. baumannii* (CRAB) and epidemiologic information

Group (N)	Phylogenetic clade	Pasteur ST (N)	Oxford ST (N)	Isolated location (N)	Isolated year (N)	Origin of bacteremia	Novel sequence
IC II	Clade 1	ST2 (93)	ST191 (59)	MICU A (15) MICU B (24) MICU C (7) MICU D (5) NCU B (1) SICU (2) CCU A (1) CCU B (2)	2009 (11) 2010 (5) 2011 (10) 2012 (11) 2013 (12) 2014 (5) 2015 (5)	Respiratory tract (46) Gastrointestinal tract (3) Musculoskeletal (1) Catheter (6) Other (2)	No
	Clade 2		ST368 (3)	MICU B (2) CCU A (1)	2009 (2) 2012 (1)	Respiratory tract (3)	Yes
	Clade 2		ST357 (7)	MICU A (5) MICU B (1) MICU D (1)	2010 (1) 2011 (3) 2012 (3)	Respiratory tract (7)	No
	Clade 3		ST208/ST1806 (5)	MICU B (2) MICU C (1) MICU D (1)	2011 (1) 2012 (3) 2013 (1)	Other (1)	Yes
	Clade 3		ST369 (3)	MICU A (1) MICU D (2)	2011 (1) 2013 (2)	Respiratory tract (2) Other (1)	No
	Clade 2		ST858 (1)	MICU C (1)	2013 (1)	Respiratory tract (1)	Yes

	Clade 4		ST451/ST1809 (11)	MICU A (6) MICU B (4) CCU A (1)	2013 (1) 2014 (6) 2015 (4)	Respiratory tract (9) Urinary tract(1) Gastrointestinal tract (1)	Yes
	Clade 1		ST784 (1)	MICU C (1)	2015 (1)	Musculoskeletal (1)	No
	Clade 1		ST191/ST784 (1)	MICU B (1)	2015 (1)	Respiratory tract (1)	Yes
Non-IC II	NA	ST193 (1)	ST552 (1)	CCU A (1)	2010 (1)	Respiratory tract (1)	No
	NA	ST10 (4)	ST447 (4)	MICU A (2) MICU B (2)	2013 (1) 2014 (3)	Respiratory tract (4)	No
NA	NA	NA	ABAY12003	MICU A (1)	2012 (1)	Respiratory tract (1)	
			ABAY13017	MICU A (1)	2013 (1)	Catheter (1)	

Data are expressed as number (N). Abbreviation: ST, sequence type; IC, international clone; MLST, Multi-locus sequence typing; MICU, medical intensive care unit; NCU, neurologic intensive care unit; SICU, surgical intensive care unit; CCU, cardiac intensive care unit; NA, not applicable

3. Core genome-based phylogeny and SNP-based inference of recombination

A core genome alignment-based phylogenetic tree was constructed to delineate the in-depth relationship between the analysed strains. The analysis resulted in the classification of 98 analysed strains into four major clades. SNP identification relied on read mapping on the reference genome sequence of the strain KBN10P02143. Pairwise SNP distances among all strains ranged from 39 to 83,304 bp (5,368 bp median) (Supplementary Table S4). Relationships between SNP-based phylogeny and STs were searched. Clade 1 was mostly composed of ST191. Majority of strains classified as clade 2 (ST368, ST357,ST858) were isolated in the former part of our study period, while those of clade 3 (ST208/ST1806, ST369) were isolated in the latter part of our study period. However, chronological correlation among clades was not identified overall.

4. Differences in patient characteristics among STs

Patients were hospitalised in different types of ICUs and the locations of each isolated ST are shown in Table 1. Most strains were identified in MICU and no notable relationship between isolated locations and STs was reported. Mean age of the enrolled patients was 60.62 ± 14.63 years and male patients predominated (70.4%, n = 69). Chronic obstructive pulmonary disease (COPD) was the most commonly found underlying morbidity (93.9%, n = 92), and chemotherapy was the most common cause of immunosuppression (39.8%, n = 39). Ventilator care was performed in 81.6% of patients (n = 80). The most common source of bacteraemia was respiratory tract (79.6%, n = 78). The median hospitalisation days were 30 [19-64.2][26] and patients used antibiotics with a median length of 30.5 [16-59.3] days. The all cause 28-day mortality was 80.6% (n = 79). No significant differences were observed in clinical characteristics among STs except the following: Patients with strains typed as ST447 spent less amount of time in hospital (17 [17-41] versus 30 [19-64.2], $p < 0.05$) and those with strains typed as ST191 had fewer males (40 [63.5%] versus 29 [82.9%], $p < 0.05$) (Table 2).

Table 2. Baseline characteristics of patients with carbapenem resistant *A.baumannii* bacteremia, grouped by sequence types

Chracteristics	Grouped by sequence type						Total
	ST191			ST447			
	ST191(N=59)	Non ST191(N=39)	p-value	ST447(n=4)	Non ST447(n=94)	p-value	
Age, years	59.27±14.70	63.13±14.39	0.21	77.0±10.74	60.09±14.41	0.05	60.62±14.63
Gender, male	**37(62.7)**	**32(84.2)**	**0.02**	3(75.0)	66(71.0)	0.67	69(70.4)
Comorbiditie							
DM	43(72.9)	32(82.1)	0.23	2(50.0)	73(77.7)	0.24	75(67.5)
COPD	55(93.2)	37(94.9)	0.56	4(100.0)	88(93.6)	0.77	92(93.9)
CHF	49(83.1)	34(87.2)	0.42	4(100.0)	79(84.0)	0.51	83(84.7)
HTN	36(61.0)	26(66.7)	0.40	2(50.0)	60(63.8)	0.47	62(63.3)
CRF	42(71.2)	32(82.1)	0.18	3(75.0)	71(75.5)	0.69	74(75.5)
Chronic liver disease	52(88.1)	37(94.8)	0.24	4(100.0)	85(90.4)	0.60	89(90.8)
Malignancy	35(59.3)	22(56.4)	0.56	2(50.0)	55(58.5)	0.67	57(58.2)
Immunosuppressed status							
Transplantation	7(11.9)	8(21/1)	0.18	0(0.0)	15(16.1)	0.50	15(15.3)
Steroid use[a]	20(33.9)	10(26.3)	0.29	0(0.0)	30(32.3)	0.22	30(30.6)
Chemotherapy	23(39.0)	16(42.1)	0.46	2(50.0)	37(39.8)	0.53	39(39.8)
APACHE II	21.32±8.16	21.26±7.18	0.97	23.25±3.50	21.22±7.881	0.35	21.17±7.81
Admission episode(≥ once)	42(71.19)	25(64.10)	0.27	3(75.0)	64(68.1)	0.62	67(68.4)
Source of infection							

Respiratory tract	46(82.1)	32(88.9)	0.29	4(100.0)	74(78.7)	0.35	78(79.6)
Other	13(22.0)	7(17.9)		0(0.0)	20(21.3)		
Time to bacteremia	21(12-41)	19(12-41)	0.38	12(8.2-37)	20.5(12-41)	0.21	20.5(12-41)
Invasive procedure							
Ventilator	47(79.7)	33(86.8)	0.27	4(100.0)	76(81.7)	0.47	80(81.6)
CRRTx	18(30.5)	11(28.9)	0.53	2(50.0)	27(29.0)	0.35	29(29.6)
C-line insertion	52(88.1)	35(92.1)	0.40	4(100.0)	83(89.2)	0.64	87(88.8)
Operation	21(35.6)	8(21.1)	0.09	0(0.0)	29(31.2)	0.24	29(29.6)
Hospital stay (days)	32(20-57)	27(17-71)	0.77	**17(17-41)**	**30(19-64.2)**	**0.04**	30(19-64.2)
ICU stay (days)	22(11-38)	19(8-52)	0.34	17(15.5-33.5)	21(9.8-40.5)	0.34	21(9.8-40.5)
Duration of antibiotics	31(17-57)	25(16-69)	0.84	16.5(16-41)	30.5(16-59.3)	0.07	30.5(16-59.3)
Duration of susceptible antibiotics	0(0-9)	8(0-17)	0.36	4.5(0.3-10.8)	2(0-11.25)	0.76	2(0-11)
Susceptible antibiotics use[b]	4(6.8)	7(18.4)	0.08	1(25)	10(10.8)	0.39	11(11.2)
All cause 28 day mortality	52(88.1)	27(71.1)	0.16	4(100.0)	75(80.6)	0.81	79(80.6)

Data of sequence types only with statistical significance are shown. Data are expressed as the mean ± SD / median (Q1-Q3) or N (%). Abbreviation: DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; CHF, congestive heart failure; HTN, hypertension; CRF, chronic renal failure; APACHE, acute physiology and chronic health evaluation; CRRTx., continuous renal replacement therapy

*signifies having statistical significance with p-value<0.05 when compared to groups of the rest

[a] Steroid use defined by use of ≥20mg prednisolone for ≥2weeks

[b] We consider antibiotics susceptible when phenotypically susceptible antibiotics were used for more than half of time after culture recovered.

5. Differences in antimicrobial resistance determinants according to STs and year of isolation

Among 29 AMR genes investigated, average 16.64 genes were possessed by each strain. $bla_{\text{OXA-23}}$ was identified in all strains except for ABAY11010 (ST369) and ABAY12016 (ST208/ST1806). Among class D carbapenem-hydrolyzing class D β-lactamase genes, $bla_{\text{OXA-82}}$ and $bla_{\text{OXA-66}}$ were found in ABAY11010 and ABAY12016 respectively.

The composition of antimicrobial resistance determinants within each ST was relatively constant, especially in ST191. As we compared the first half of research period with the latter half, only *strA* (0%, n = 0 versus 18.2%, n = 4; p = 0.02) showed increased prevalence. Furthermore, we observed a decrease in the prevalence of *aac*(3)-Ia (9.1%, n = 2 versus 91.9%, n = 34; p < 0.01), *armA* (86.4%, n = 19 versus 100%, n = 37; p = 0.04), and $bla_{\text{TEM-1D}}$ (18.2%, n = 4 versus 97.3%, n = 36; p < 0.01). The comparison of the source of bacteraemia showed that *aadA1b* was the most common in the isolates from the respiratory tract than in any other sources (51.9%, n = 14 versus 0%, n = 0; p < 0.01) (Table 4).

However, we observed clear distinction in composition of AMR genes among ST types (Table 3). Most ST447 and ST451/ST1809 that were the dominant STs in the latter part of our study had least amount of AMR genes. *aac(6')-Ib, aac(6')-Ib, aadA1, ant(3'')-Ia,* and *sul1* were less frequently detected in these types, while *sul*2, and *tet(B)* were the genes more frequently observed. The ST that contained the most abundant AMR genes was ST208/ST1806. This ST possessed class A extended-spectrum β-lactamase gene $bla_{\text{PER-1}}$, which is a rare contributor of antimicrobial resistance in *A.baumannii* , among others(Table 3).

The comparison of the total number of AMR genes based on the year of isolation showed fluctuating results. The strains isolated in 2012 exhibited the most abundant AMR genes, while those found in 2014 exhibited the least amount of these genes. *aac(3)-Ia, aac(6')-Ib, aac(6')-Ib3 aadA1, ant(3'')-Ia ,blaTEM-1D, mph(E), sul1* and *aac(6')-Ib-cr* were significantly decreased in the latter part of study period, while *strA* and *tet(B)* were increased. The change was not significant upon separation and chronological comparison of only ST191 (Fig. 2A, 2B).

Table 3. Composition of antimicrobial resistant genes according to sequence types

Gene	Sequence types											
	ST191 (n=59)	ST357 (n=7)	ST368 (n=3)	ST208/ST1806 (n=5)	ST552 (n=1)	ST858 (n=1)	ST369 (n=3)	ST784 (n=1)	ST191/ST784 (n=1)	ST451/ST1809 (n=11)	ST447 (n=4)	NA (n=2)
Aminoglycoside												
aac(3)-Ia	**36(61.0)**	**0(0)**	3(100)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	**0(0.0)**	0(0.0)	2(100)
aac(6')-Iaf	0(0.0)	0(0)	**2(66.7)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
aac(6')-Ib	**57(96.6)**	7(100)	1(33.3)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	**0(0.0)**	**1(25.0)**	2(100)
aac(6')-Ib3	**57(96.6)**	7(100)	1(33.3)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	**0(0.0)**	**1(25.0)**	2(100)
aadA1	**58(98.3)**	7(100)	1(33.3)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	**0(0.0)**	**1(25.0)**	2(100)
aadA1b	29(50.0)	2(28.6)	3(100)	4(80.0)	0(0.0)	0(0.0)	1(33.3)	1(100)	1(100)	**0(0.0)**	1(25.0)	0(0.0)
ant(3'')-Ia	**57(96.6)**	7(100)	1(33.3)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	**0(0.0)**	**1(25.0)**	2(100)
aph(3')-Ia	**0(0.0)**	3(42.9)	0(0.0)	**4(80.0)**	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)	**7(63.6)**	0(0.0)	0(0.0)
aph(3'')-Ib	**0(0.0)**	**7(100)**	2(66.7)	**5(100)**	0(0.0)	1(100)	**3(100)**	0(0.0)	0(0.0)	**11(100)**	0(0.0)	0(0.0)
aph(6)-Id	**0(0.0)**	**7(100)**	2(66.7)	**5(100)**	0(0.0)	1(100)	**3(100)**	0(0.0)	0(0.0)	**11(100)**	0(0.0)	0(0.0)
aph(3')-VIb	**0(0.0)**	0(0)	0(0.0)	**4(80.0)**	0(0.0)	(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
aph(3')-Vij	1(1.7)	0(0)	0(0.0)	**4(80.0)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
armA	**56(94.9)**	7(100)	**1(33.3)**	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	10(90.9)	**1(25.0)**	2(100)
strA	**4(6.8)**	**7(100)**	2(66.7)	**5(100)**	0(0.0)	1(100)	2(66.7)	0(0.0)	0(0.0)	**11(100)**	1(25.0)	1(50.0)
b-lactam												
blaADC-25	59(100)	7(100)	3(100)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	11(100)	**0(0.0)**	2(100)
blaOXA-23	58(98.3)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
blaOXA-66	**59(100)**	7(100)	3(100)	5(100)	0(0.0)	1(100)	**1(33.3)**	1(100)	1(100)	11(100)	**1(25.0)**	2(100)
blaOXA-68	0(0.0)	0(0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**3(75.0)**	0(0.0)
blaOXA-82	0(0.0)	0(0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**2(66.7)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
blaOXA-120	1(1.7)	0(0)	0(0.0)	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
blaPER-1	0(0.0)	0(0)	0(0.0)	**4(80.0)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
blaTEM-1D	40(67.8)	5(71.4)	3(100)	3(60)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	7(63.6)	**0(0.0)**	1(50.0)
Microlide												

mph(E)	50(84.7)	7(100)	1(33.3)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	10(90.9)	**0(0.0)**	1(50.0)
msr(E)	53(89.8)	7(100)	**1(33.3)**	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	10(90.9)	**1(25.0)**	2(100)
Sulphonamide												
sul1	**57(96.6)**	7(100)	1(33.3)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	**0(0.0)**	**1(25.0)**	2(100)
sul2	**0(0.0)**	6(85.7)	2(66.7)	**5(100)**	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	**4(100)**	0(0.0)
Chloramphenicol												
catB8	**56(94.9)**	7(100)	0(0.0)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	**0(0.0)**	**1(25.0)**	2(100)
Quinolone												
aac(6')-Ib-cr	**52(88.1)**	7(100)	1(33.3)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	0(0.0)	**1(25.0)**	2(100)
Tetracycline												
tet(B)	**0(0.0)**	**7(100)**	2(66.7)	1(20)	0(0.0)	1(100)	2(66.7)	0(0.0)	0(0.0)	**11(100)**	**4(100)**	0(0.0)

Data are expressed as N (%). Values with statistical significance (p-value<0.05) when compared to groups of the rest were expressed in boldface. Abbreviation: ST, sequence type; NA, not applicable

Table 4. Composition of antimicrobial resistant genes of all sequence types combined and sequence type 191 according to isolation date or source of bacteremia

Gene	ST191						All		
	Source of bacteremia			Isolated year			Isolated year		
	Respiratory tract(n=46)	Other(n=13)	p-value	2009-2012(n=37)	2013-2015(n=22)	p-value	2009-2012(n=54)	2013-2015(n=44)	p-value
Aminoglycoside									
aac(3)-Ia	29(63.0)	7(53.8)	0.39	**34(91.9)**	**2(9.1)**	**<0.01**	**38(70.4)**	**3(6.8)**	**<0.01**
aac(6')-Iaf	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	2(3.7)	0(0)	0.30
aac(6')-Ib	44(95.7)	13(100)	0.60	36(97.3)	21(95.5)	0.61	**50(92.6)**	**29(65.9)**	**<0.01**
aac(6')-Ib3	44(95.7)	13(100)	0.60	37(100)	20(90.9)	0.14	**51(94.4)**	**28(63.6)**	**<0.01**
aadA1	45(97.8)	13(100)	0.78	37(100)	21(95.5)	0.37	**51(94.4)**	**29(65.9)**	**<0.01**
aadA1b	**14(51.9)**	**0(0.0)**	**<0.01**	10(27.0)	4(18.1)	0.57	30(55.6)	17(68.6)	0.07
ant(3'')-Ia	44(95.7)	13(100)	0.60	37(100)	20(90.9)	0.14	**51(94.4)**	**28(63.6)**	**<0.01**
aph(3')-Ia	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	6(11.1)	9(20.5)	0.16
aph(3'')-Ib	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	14(25.9)	15(34.1)	0.26
aph(6)-Id	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	14(25.9)	15(34.1)	0.26
aph(3')-VIb	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	3(5.6)	1(2.3)	0.39
aph(3')-Vij	1(2.2)	0(0.0)	0.78	1(2.7)	0(0.0)	0.63	4(7.4)	1(2.3)	0.25
armA	43(93.5)	13(100)	0.47	**37(100)**	**19(86.4)**	**0.04**	51(94.4)	37(84.1)	0.09
strA	4(8.7)	0(0.0)	0.36	**0(0.0)**	**4(18.2)**	**0.02**	**14(25.9)**	**21(47.7)**	**0.02**
b-lactam									
blaADC-25	46(100)	13(100)	1.00	37(100)	22(100)	1.00	53(98.1)	40(90.9)	0.12
blaOXA-23	45(97.8)	13(100)	0.78	37(100)	21(95.5)	0.37	53(98.1)	43(97.7)	0.69
blaOXA-66	46(100)	13(100)	1.00	37(100)	22(100)	1.00	52(96.3)	39(90.7)	0.24
blaOXA-68	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	0(0)	3(6.8)	0.09
blaOXA-82	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	1(1.9)	2(4.5)	0.42
blaOXA-120	1(2.2)	0(0.0)	0.78	1(2.7)	0(0.0)	0.67	1(1.9)	0(0)	0.55
blaPER-1	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	3(5.6)	1(2.3)	0.39
blaTEM-1D	33(71.7)	7(53.8)	0.19	**36(97.3)**	**4(18.2)**	**<0.01**	**47(87.0)**	**12(27.3)**	**<0.01**

Microlide									
mph(E)	37(80.4)	13(100)	0.08	35(94.6)	15(68.2)	0.01	**49(90.7)**	**31(70.5)**	**0.01**
msr(E)	41(89.1)	12(92.3)	0.60	34(91.9)	19(86.4)	0.40	48(88.9)	37(84.1)	0.34
Sulphonamide									
sul1	45(97.8)	12(92.3)	0.40	37(100)	20(90.9)	0.14	**51(94.4)**	**28(63.6)**	**<0.01**
sul2	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	12(22.2)	17(38.6)	0.06
Chloramphenicol									
catB8	44(95.7)	12(92.3)	0.53	35(94.6)	21(95.5)	0.69	**49(90.7)**	**29(65.9)**	**<0.01**
Quinolone									
aac(6')-Ib-cr	40(86.9)	12(92.3)	0.35	45(97.8)	17(81.0)	0.12	**50(92.6)**	**29(65.9)**	**<0.01**
Tetracycline									
tet(B)	0(0.0)	0(0.0)	1.00	0(0.0)	0(0.0)	1.00	**11(20.4)**	**18(40.9)**	**0.02**

Data are expressed as N (%).Values with statistical significance (p-value<0.05) were expressed in boldface

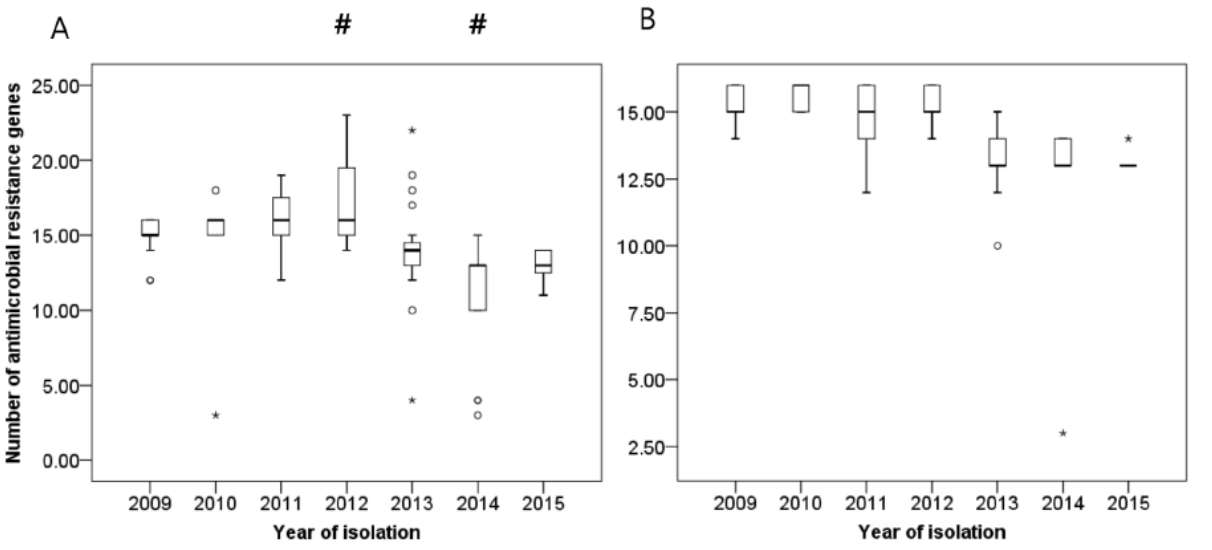

Figure 2. Changes in numbers of antimicrobial resistance genes according to year of isolation. The bar graph represents the medians, percentiles (25^{th} and 75^{th}) and 95% confidence intervals: (A) when all sequence types were included and (B) when only sequence type 191 was included.

Circle (°) and asterisk (*) symbols indicate extreme values.

Pound (#) indicates a statistically significant result ($p<0.05$) when compared to the rest of the year.

6. Differences in virulence genes according to STs and year of isolation

From the literature survey, we compiled a list of 182 potential virulence genes [27]. Of all genes identified, 107 genes were possessed by all strains isolated. Most differences among STs were found in ACICU_00075-ACICU_00087 (Immune evasion), *bap*, *csuA*, *casuA/B*, *csuB*, *csuC*, *csuD*, *csuE*, *pgaD* (biofilm formation), and *vgrG1*, *vgrG2*, and *vgrG4* (Type IV protein secretion system) genes (Table 5). We separated ST191 and searched for epidemiologic differences to find that only *carO* related to porin was more frequently detected in the samples originating from the respiratory tract. No statistically significant chronological changes were observed (Table 6). The ST that possessed the most abundant virulence genes was ST208/ST1809, which had more ACICU_00075-ACICU_00087, *pgaD*, bla_{PER-1}, and *hcp* genes than others. The second most common type was ST451/ST1809, which possessed more ACICU_00075-ACICU_00080, ACICU_00086, ACICU_00087, *pgaD*, *pclC1*, and *hcp*. ST552 had the least amount of virulence genes and was devoid of bla_{OXA-24}, ABUW_1966, ACICU_00074-ACICU_00087, *fhaC*, *pgaD*, bla_{PER-1}, *omp33-36*, *plcC1*, and *hcp*. The ST that had the

second least amount of virulence genes was ST447 (Table 5).

The number of virulence genes was compared according to the year of isolation. The number increased as the research period passed toward the end. The strains isolated in the year 2009 carried fewer virulence genes, while those isolated in 2015 exhibited more virulence genes than the rest. We singled out ST191 and observed statistically insignificant results (Fig. 3A, 3B).

Table 5. Composition of virulence genes according to sequence types

Gene	Sequence types											
	ST191 (n=59)	ST357 (n=7)	ST368 (n=3)	ST208/ST1806 (n=5)	ST552 (n=1)	ST858 (n=1)	ST369 (n=3)	ST784 (n=1)	ST191/ST784 (n=1)	ST451/ST1809 (n=11)	ST447 (n=4)	NA (n=2)
Outer membrane vesicle												
A1S_0009	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_0116	**59(100)**	7(100)	**0(0.0)**	5(100)	1(100)	0(0.0)	0(0.0)	1(100)	1(100)	11(100)	**0(0.0)**	2(100)
A1S_1180	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_1321	58(98.3)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_1386	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_1510	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_1921	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_2470	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_2525	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_3143	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_3175	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
A1S_3411	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ACV72173.1	56(94.9)	6(85.7)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	1(50)
ACV72174.1	56(94.9)	7(100)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	1(50)
ADB23465.1	55(93.2)	7(100)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	1(50)
ADB23466.1	51(86.4)	5(71.4)	1(33.3)	**2(40.0)**	1(100)	1(100)	3(100)	1(100)	1(100)	10(90.9)	4(100)	1(50)
ADB23467.1	55(93.2)	7(100)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	0(0.0)	11(100)	4(100)	1(50)
ADB23468.1	56(94.9)	7(100)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	1(50)
ADB23470.1	56(94.9)	7(100)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	1(50)
ADB23471.1	53(89.8)	6(85.7)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	1(50)
ADB23472.1	49(83.1)	7(100)	1(33.3)	4(80)	1(100)	1(100)	2(66.7)	1(100)	1(100)	11(100)	4(100)	1(50)
ADB23473.1	55(93.2)	7(100)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	1(100)	9(81.8)	4(100)	1(50)
GADB23474.1	56(94.9)	6(85.7)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	1(50)
GADB23475.1	56(94.9)	7(100)	**1(33.3)**	4(80)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	1(50)
$bla_{\text{OXA-24}}$	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Antibiotic resistance												
ABUW_1156	**59(100)**	7(100)	3(100)	5(100)	1(100)	0(0.0)	3(100)	1(100)	1(100)	11(100)	**0(0.0)**	2(100)
ABUW_1499	**56(94.9)**	**0(0.0)**	3(100)	5(100)	1(100)	1(100)	1(33.3)	1(100)	1(100)	11(100)	2(50)	2(100)
ABUW_1520	**54(91.5)**	**0(0.0)**	3(100)	5(100)	1(100)	1(100)	1(33.3)	1(100)	1(100)	**0(0.0)**	2(100)	2(100)
ABUW_1645	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_1672	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_1673	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_1692	58(98.3)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_1755	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_1768	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_1849	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_1851	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_1966	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
ABUW_2074	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(00)	11(100)	4(100)	2(100)
ABUW_2550	58(98.3)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(00)	11(100)	4(100)	2(100)
Aromatic hydrocarbon metabolism												
ABUW_2090	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_2123	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_2236	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_2349	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_2370	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ABUW_2374	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
benP1	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
paaI1	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
paaY	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
pcaC	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
pcaD1	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
pcaU	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)

Transcriptional regulation												
ABUW_2520	59(100)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(00)	1(00)	11(100)	4(100)	2(100)
ABUW_2544	58(98.3)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(00)	1(00)	11(100)	4(100)	2(100)
Immune evasion												
ACICU_00074	59(100)	7(100)	3(100)	5(100)	0(0.0)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ACICU_00075	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	**0(0.0)**	0(0.0)
ACICU_00076	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	**0(0.0)**	0(0.0)
ACICU_00077	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	**0(0.0)**	0(0.0)
ACICU_00078	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	**0(0.0)**	0(0.0)
ACICU_00079	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	**0(0.0)**	0(0.0)
ACICU_00080	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	**0(0.0)**	0(0.0)
ACICU_00081	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**0(0.0)**	0(0.0)
ACICU_00082	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**0(0.0)**	0(0.0)
ACICU_00083	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**0(0.0)**	0(0.0)
ACICU_00084	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**0(0.0)**	0(0.0)
ACICU_00085	**0(0.0)**	0(0.0)	0(0.0)	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**0(0.0)**	0(0.0)
ACICU_00086	**0(0.0)**	0(0.0)	**3(100)**	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	**0(0.0)**	0(0.0)
ACICU_00087	**0(0.0)**	0(0.0)	**3(100)**	**5(100)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	**0(0.0)**	0(0.0)
ACICU_00088	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ACICU_00089	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ACICU_00091	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ACICU_00092	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
lpsB	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
lptE	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
lpxA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
lpxB	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
lpxC	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
lpxD	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)

lpxL	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
lpxM	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
eps	**59(100)**	**0(0.0)**	**0(0.0)**	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	
pgi	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ptk	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ptp	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Regulation												
abaI	**59(100)**	7(100)	**0(0.0)**	5(100)	1(100)	0(0.0)	3(100)	1(100)	1(100)	11(100)	**0(0.0)**	2(100)
abaR	**54(91.5)**	7(100)	**0(0.0)**	5(100)	1(100)	0(0.0)	3(100)	1(100)	1(100)	11(100)	**0(0.0)**	2(100)
bfmR	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
bfmS	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Killing of host cells												
abeD	59(100)	7(100)	3(100)	5(100)	1(100)	0(0.0)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
envZ	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
fhaB	**58(98.3)**	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	9(81.8)	**0(0.0)**	1(50)
fhaC	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Biofilm formation												
adeF	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
adeG	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
bap	**59(100)**	7(100)	3(100)	5(100)	1(100)	0(0.0)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
csuA	**57(96.6)**	**0(0.0)**	3(100)	5(100)	1(100)	0(0.0)	1(33.3)	1(100)	1(100)	10(90.9)	**1(25)**	2(100)
csuA/B	**57(96.6)**	**0(0.0)**	3(100)	5(100)	1(100)	0(0.0)	1(33.3)	1(100)	1(100)	9(81.8)	**1(25)**	2(100)
csuB	**57(96.6)**	**0(0.0)**	3(100)	5(100)	1(100)	0(0.0)	1(33.3)	1(100)	1(100)	9(81.8)	**1(25)**	2(100)
csuC	**57(96.6)**	**0(0.0)**	3(100)	5(100)	1(100)	0(0.0)	1(33.3)	1(100)	1(100)	10(90.9)	**1(25)**	2(100)
csuD	**57(96.6)**	**0(0.0)**	3(100)	5(100)	1(100)	0(0.0)	1(33.3)	1(100)	1(100)	10(90.9)	**1(25)**	2(100)
csuE	**56(94.9)**	**0(0.0)**	3(100)	5(100)	1(100)	0(0.0)	1(33.3)	1(100)	1(100)	10(90.9)	**1(25)**	2(100)
pgaA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)

pgaB	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
pgaC	**59(100)**	7(100)	3(100)	5(100)	1(100)	0(0.0)	3(100)	1(100)	1(100)	11(100)	**0(0.0)**	2(100)
pgaD	**0(0.0)**	**7(100)**	2(66.7)	**5(100)**	0(0.0)	1(100)	**3(100)**	0(0.0)	0(0.0)	**11(100)**	**4(100)**	0(0.0)
adeH	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Antibiotic resistance												
adeI	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
adeJ	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
adeK	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
$bla_{\text{PER-1}}$	**0(0.0)**	0(0.0)	0(0.0)	**4(80.0)**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Transcriptional regulation												
alkR	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
gigA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
gigB	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
gigC	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
soxR	58(98.3)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Iron uptake												
barA	59(100)	7(100)	3(100)	5(100)	1(100)	0(0.0)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
barB	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
basA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
basB	59(100)	7(100)	3(100)	5(100)	1(100)	0(0.0)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
basC	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
basD	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
basF	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
basG	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
basH	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
basI	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
basJ	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)

bauA	**59(100)**	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	**0(0.0)**	2(100)
bauB	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
bauC	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
bauD	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
bauE	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
bauF	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
nfuA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Porin												
carO	57(96.6)	7(100)	3(100)	5(100)	1(100)	1(100)	**1(33.3)**	1(100)	1(100)	11(100)	4(100)	2(100)
omp22	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
omp33-36	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
ompR	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
orpD	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Serun resistance, invasion												
cipA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
cobA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
pbpG	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
surA1	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
tuf	58(98.3)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
typA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Enzyme												
plcC1	**0(0.0)**	**0(0.0)**	0(0.0)	0(0.0)	0(0.0)	1(00)	0(0.0)	0(0.0)	0(0.0)	**11(100)**	0(0.0)	0(0.0)
plcC2	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
plcD	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
pldA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Cysteine												

metabolism												
cysD	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
cysE	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
cysH	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
cysI	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
cysN	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
cysQ	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
sulP	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Siderophore biosynthesis												
entA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Neutrophil influx												
gacS	59(100)	7(100)	3(100)	5(100)	1(100)	0(0.0)	3(100)	1(00)	1(00)	**9(81.8)**	4(100)	2(100)
paaA	58(98.3)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Type II protein secretion system												
gspD	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
Type VI protein secretion system												
vgrG1	**59(100)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	1(100)	0(0.0)	3(100)	1(100)	1(100)	**0(0.0)**	**0(0.0)**	2(100)
vgrG2	**59(100)**	7(100)	**1(33.3)**	5(100)	1(100)	1(00)	3(100)	1(100)	1(100)	**0(0.0)**	4(100)	2(100)
vgrG3	59(100)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
vgrG4	**59(100)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	1(100)	0(0.0)	3(100)	1(100)	1(100)	**0(0.0)**	**0(0.0)**	2(100)
hcp	**0(0.0)**	**7(100)**	0(0.0)	**5(100)**	0(0.0)	0(0.0)	**3(100)**	0(0.0)	1(100)	**11(100)**	0(0.0)	0(0.0)

Type V protein secretion system												
ata	**53(89.8)**	**2(28.6)**	3(100)	5(100)	1(100)	1(00)	3(100)	1(00)	1(00)	11(100)	4(100)	1(50)
Stress response genes												
kef	59(100)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(00)	0(0.0)	11(100)	4(100)	2(100)
kefF	58(98.3)	7(100)	**1(33.3)**	4(80)	1(100)	1(00)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
mscS	59(100)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
ostB	59(100)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
recA	59(100)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
resP	59(100)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
trkH	59(100)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
upsA	59(100)	7(100)	3(100)	5(100)	1(100)	1(00)	3(100)	1(100)	1(100)	11(100)	4(100)	2(100)
uspA	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
uvrD	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Manganese acquisition system												
mumC	58(98.3)	7(100)	3(100)	4(80)	1(100)	1(00)	3(100)	1(00)	1(00)	11(100)	4(100)	2(100)
mumT	58(98.3)	7(100)	3(100)	4(80)	1(100)	1(00)	3(100)	1(00)	1(00)	11(100)	4(100)	2(100)
Adherence												
ompA	59(100)	7(100)	3(100)	5(100)	1(010)	1(00)	3(100)	1(00)	1(00)	11(100)	4(100)	2(100)
Micronutrient acquisition												
znuA	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	1(100)	1(100)	1(100)	11(100)	4(100)	2(100)
znuB	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	1(100)	1(100)	1(100)	11(100)	4(100)	2(100)
znuC	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	1(100)	1(100)	1(100)	11(100)	4(100)	2(100)

zur	59(100)	7(100)	3(100)	5(100)	1(100)	1(100)	1(100)	1(100)	1(100)	11(100)	4(100)	2(100)

Data are expressed as N (%).Values with statistical significance (p-value<0.05) when compared to groups of the rest were expressed in boldface. Abbreviation: ST, sequence type; NA, not applicable

Table 6. Composition of virulence genes of all sequence types combined and sequence type 191 according to isolation date or source of bacteremia

Gene	ST191						ALL		
	Source of bacteremia			Isolated year			Isolated year		
	Respiratory tract(n=46)	Other(n=13)	p-value	2009-2012(n=37)	2013-2015(n=22)	p-value	2009-2012(n=37)	2009-2012(n=37)	p-value
Outer membrane vesicle									
A1S_0009	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
A1S_0116	46(10)	13(100)	1	37(100)	22(100)	1	50(92.6)	39(88.6)	0.37
A1S_1180	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
A1S_1321	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	53(98.1)	44(100)	0.55
A1S_1386	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
A1S_1510	46(10)	13(100)	1	37(100)	22(100)	1	53(98.1)	44(100)	0.55
A1S_1921	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
A1S_2470	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
A1S_2525	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
A1S_3143	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
A1S_3175	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
A1S_3411	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ACV72173.1	43(93.5)	13(100)	0.47	34(91.9)	22(100)	0.24	**46(85.2)**	**43(97.7)**	**0.03**
ACV72174.1	43(93.5)	13(100)	0.47	34(91.9)	22(100)	0.24	47(87.0)	43(97.7)	0.06
ADB23465.1	43(93.5)	13(100)	0.47	34(91.9)	21(95.5)	0.52	47(87.0)	43(97.7)	0.06
ADB23466.1	39(84.8)	12(92.3)	0.43	31(83.8)	20(90.9)	0.36	40(	39(	
ADB23467.1	43(93.5)	12(92.3)	0.64	34(91.9)	21(95.5)	0.52	47(87.0)	42(95.5)	0.14
ADB23468.1	43(93.5)	13(100)	0.47	34(91.9)	22(100)	0.24	47(87.0)	43(97.7)	0.06
ADB23470.1	43(93.5)	13(100)	0.47	34(91.9)	22(100)	0.24	47(87.0)	43(97.7)	0.06
ADB23471.1	40(87.0)	13(100)	0.21	33(89.2)	20(90.9)	0.60	45(83.3)	41(93.2)	0.126
ADB23472.1	37(80.4)	12(92.3)	0.29	28(75.7)	21(95.5)	0.07	**41(75.9)**	**40(90.9)**	**0.04**

ADB23473.1	42(91.3)	13(100)	0.36	33(89.2)	22(100)	0.15	**46(85.2)**	**43(97.7)**	**0.03**
GADB23474.1	43(93.5)	13(100)	0.47	34(91.9)	22(100)	0.24	**46(85.2)**	**43(97.7)**	**0.03**
GADB23475.1	43(93.5)	13(100)	0.47	34(91.9)	22(100)	0.24	47(87.0)	43(97.7)	0.06
bla$_{OXA-24}$	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	0(0)	0(0)	1
Antibiotic resistance									
ABUW_1156	46(10)	13(100)	1	37(100)	22(100)	1	53(98.1)	40(90.9)	0.12
ABUW_1499	43(93.5)	13(100)	0.47	36(97.3)	20(90.9)	0.31	45(83.3)	38(86.4)	0.45
ABUW_1520	41(89.2)	13(100)	0.27	35(94.6)	19(86.4)	0.26	**45(83.3)**	**26(59.1)**	**<0.01**
ABUW_1645	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_1672	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_1673	46(10)	13(100)	1	37(100)	22(100)	1	53(98.1)	44(100)	0.55
ABUW_1692	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	53(98.1)	44(100)	0.55
ABUW_1755	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_1768	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_1849	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_1851	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_1966	46(10)	13(100)	1	37(100)	22(100)	1	0(0)	0(0)	1
ABUW_2074	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_2550	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	53(98.1)	44(100)	0.55
Aromatic hydrocarbon metabolism									
ABUW_2090	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_2123	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_2236	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_2349	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_2370	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_2374	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
benP1	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1

paaI1	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
paaY	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
pcaC	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
pcaD1	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
pcaU	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
Transcriptional regulation									
ABUW_2520	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ABUW_2544	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	52(96.3)	44(100)	0.30
Immune evasion									
ACICU_00074	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ACICU_00075	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	**4(7.4)**	**12(27.3)**	**<0.01**
ACICU_00076	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	**4(7.4)**	**12(27.3)**	**<0.01**
ACICU_00077	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	**4(7.4)**	**12(27.3)**	**<0.01**
ACICU_00078	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	**4(7.4)**	**12(27.3)**	**<0.01**
ACICU_00079	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	**4(7.4)**	**12(27.3)**	**<0.01**
ACICU_00080	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	**4(7.4)**	**12(27.3)**	**<0.01**
ACICU_00081	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	4(7.4)	1(2.3)	0.25
ACICU_00082	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	4(7.4)	1(2.3)	0.25
ACICU_00083	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	4(7.4)	1(2.3)	0.25
ACICU_00084	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	4(7.4)	1(2.3)	0.25
ACICU_00085	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	4(7.4)	1(2.3)	0.25
ACICU_00086	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	**7(13.0)**	**15(34.1)**	**0.01**
ACICU_00087	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	**7(13.0)**	**15(34.1)**	**0.01**
ACICU_00088	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ACICU_00089	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ACICU_00091	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ACICU_00092	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
lpsB	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1

lptE	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
lpxA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
lpxB	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
lpxC	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
lpxD	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
lpxL	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
lpxM	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
eps	46(10)	13(100)	1	37(100)	22(100)	1	**43(79.6)**	**42(95.5)**	**0.02**
pgi	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ptk	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ptp	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
Regulation									
abaI	46(10)	13(100)	1	37(100)	22(100)	1	50(92.6)	39(88.6)	0.37
abaR	43(93.5)	11(84.6)	0.30	36(97.3)	18(81.8)	0.06	49(90.7)	35(79.5)	0.10
bfmR	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
bfmS	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
Killing of host cells									
abeD	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
envZ	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
fhaB	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	**52(96.3)**	**37(84.1)**	**0.04**
fhaC	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	1(1.9)	0(0)	0.55
Biofilm formation									
adeF	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
adeG	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
bap	46(10)	13(100)	1	37(100)	22(100)	1	53(98.1)	40(90.9)	0.12
csuA	44(95.7)	13(100)	0.61	37(100)	20(90.9)	0.14	47(87.0)	36(81.8)	0.33
csuA/B	44(95.7)	13(100)	0.61	37(100)	20(90.9)	0.14	47(87.0)	35(79.5)	0.23

csuB	44(95.7)	13(100)	0.61	37(100)	20(90.9)	0.14	47(87.0)	35(79.5)	0.23
csuC	44(95.7)	13(100)	0.61	37(100)	20(90.9)	0.14	47(87.0)	36(81.8)	0.33
csuD	44(95.7)	13(100)	0.61	37(100)	20(90.9)	0.14	47(87.0)	36(81.8)	0.33
csuE	44(95.7)	13(100)	0.61	36(97.3)	20(90.9)	0.31	46(85.2)	36(81.8)	0.43
pgaA	46(10)	13(100)	1	37(100)	22(100)	1	53(98.1)	44(100)	0.55
pgaB	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
pgaC	46(10)	13(100)	1	37(100)	22(100)	1	**54(100)**	**40(90.9)**	**0.04**
pgaD	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	15(27.8)	19(43.2)	0.14
adeH	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
Antibiotic resisance									
adeI	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
adeJ	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
adeK	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
$bla_{\text{PER-1}}$	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	3(5.6)	1(2.3)	0.39
Transcriptional regulation									
alkR	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
gigA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
gigB	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
gigC	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
soxR	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	53(98.1)	44(100)	0.55
Iron uptake									
barA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
barB	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
basA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
basB	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
basC	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
basD	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1

basF	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
basG	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
basH	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
basI	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
basJ	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
bauA	46(10)	13(100)	1	37(100)	22(100)	1	**54(100)**	**40(90.9)**	**0.04**
bauB	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
bauC	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
bauD	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
bauE	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
bauF	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
nfuA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
Porin									
carO	**46(100)**	**11(84.6)**	**0.046**	37(100)	20(90.9)	0.14	**54(100)**	**40(90.9)**	**0.04**
omp22	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
omp33-36	0(0.0)	0(0.0)	1	37(100)	22(100)	1	0	0	
ompR	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
orpD	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
Serun resistance, invasion									
cipA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
cobA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
pbpG	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
surA1	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
tuf	46(10)	13(100)	1	36(97.3)	22(100)	0.63	53(98.1)	44(100)	0.55
typA	46(10)	13(100)	1	37(100)	22(100)	1			
Enzyme									
plcC1	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	8(14.8)	14(31.8)	0.05

plcC2	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
plcD	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
pldA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
Cysteine metabolism									
cysD	46(10)	13(100)	1				54(100)	44(100)	1
cysE	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
cysH	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
cysI	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
cysN	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
cysQ	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
sulP	46(10)	13(100)	1	37(100)	22(100)	1			
Siderophore biosynthesis									
entA	46(100)	13(100)	1	37(100)	22(100)	1	54(100)	42(95.5)	0.20
Neutrophil influx									
gacS	46(10)	13(100)	1				54(100)	44(100)	1
paaA	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	53(98.1)	44(100)	0.55
Type II protein secretion system									
gspD	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
Type VI protein secretion system									
vgrG1	46(10)	13(100)	1	37(100)	22(100)	1	39(72.2)	27(61.4)	0.29

vgrG2	46(10)	13(100)	1	37(100)	22(100)	1	**52(96.3)**	**33(75.0)**	**<0.01**
vgrG3	46(10)	13(100)	1	37(100)	22(100)	1	53(98.1)	44(100)	0.55
vgrG4	46(10)	13(100)	1	37(100)	22(100)	1	40(74.1)	27(61.4)	0.20
hcp	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	12(22.2)	15(34.1)	0.14
Type V protein secretion system									
ata	41(89.1)	12(92.3)	0.60	31(83.8)	22(100)	0.08	42(77.8)	39(88.6)	0.13
Stress response genes									
kef	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
kefF	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	54(100)	44(100)	1
mscS	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
ostB	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
recA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
resP	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
trkH	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
upsA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
uspA	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	0(0)	0(0)	1
uvrD	0(0.0)	0(0.0)	1	0(0.0)	0(0.0)	1	0(0)	0(0)	1
Manganese acquisition system									
mumC	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	52(96.3)	44(100)	0.30
mumT	45(97.8)	13(100)	0.78	36(97.3)	22(100)	0.63	52(96.3)	44(100)	0.30
Adherence									
ompA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1

Micronutrient acquisition									
znuA	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
znuB	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
znuC	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1
zur	46(10)	13(100)	1	37(100)	22(100)	1	54(100)	44(100)	1

Data are expressed as N (%).Values with statistical significance (p-value<0.05) were expressed in boldface

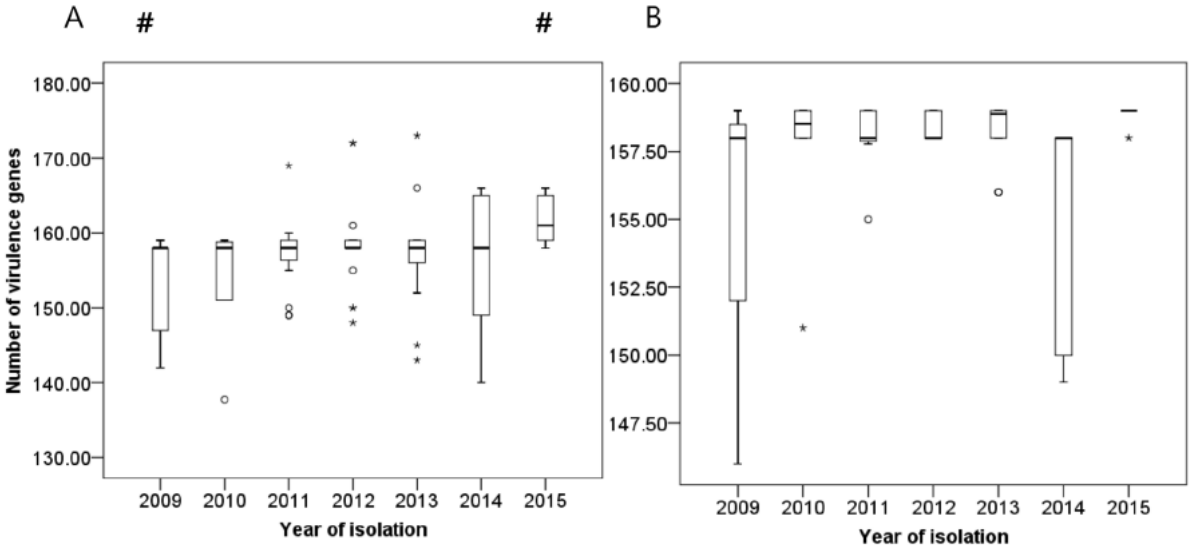

Figure 3. Changes in numbers of virulence genes according to year of isolation. The bar graph represents medians, percentiles (25th and 75th) and 95% confidence intervals: (A) when all sequence types were included and (B) when only sequence type 191 was included.

Circle (○) and asterisk (*) symbols indicate extreme values.

Pound (#) indicates a statistically significant result ($p<0.05$) when compared to the rest of the year.

7. Difference in IS*Aba1* and carbapenemase-encoding transposons on CRAB

IS*Aba1* belongs to IS*4* family and has been detected upstream of *ampC*, *bla*$_{OXA-23}$, *bla*$_{OXA-27}$, and *sul*2 antibiotic resistance genes in *Acinetobacter* species [21]. Insertion sites of IS*Aba1* were different for each strain, and no common insertion sites were conserved throughout all strains. Insertion sites of IS*Aba1* which was shared by more than 70% of strains were demonstrated as IS*1, IS*2, IS*3, IS*4, IS*5, IS*6, and IS*7. Composition of IS*1-*7 was different between STs (Supplementary Table S6). ST447 contained lower insertion sequences than the rest, while ST191 carried comparatively more insertion sequences; these incidences remained constant throughout the study period for each insertion sequence (Supplementary Fig. S2). We could detect common *bla*$_{OXA-23}$-ΔATPase modules from all isolates with the exception of ABAY11010 (ST369) and ABAY12016 (ST208/ST1806), implying that Tn*2006*, Tn*2008*, or Tn*2009* was widespread among these strains. ABAY11010 and ABAY12016

originated in the respiratory tract and had more copies of virulence and AMR genes than average (virulence genes, 160 and 161 copies each; AMR genes, 16 and 23 copies each). Both strains were isolated at different places (MICU D and MICU C each) and time (2011 and 2012 each).

8. Resistance islands

We searched contig sequences using the 87.7-kb AbaR1 sequence from the strain AYE (CU459141.1) [28] as query, and found 70 strains to carry a very short AbaR-like sequence. These were only 10,641 bp long after including the disrupted ATPase gene (*comM'*) at both ends. The AbaR-like islands were similar with 14.5-kb long Tn*6021* that is devoid of MARR (multiple antibiotic resistance region) found from ATCC 17978 (GenBank CP00521.1). When read mapping on the sequence extracted from ABAY09001 applied, all strains except ABAY13016, ABAY14002, ABAY14004, and ABAY14008 possessed AbaR-like island. These strains were categorised as ST447.

IV. DISCUSSION

Since the 1970s, there has been a progressive increase in the antimicrobial resistance of the majority of *A. baumannii* strains, which were otherwise sensitive to the commonly used antibiotics. By 2007, up to 70% of isolates in certain settings had developed multidrug resistance including resistance to carbapenems, which were once considered as the mainstay against multidrug-resistant *A. baumannii* infections [29]. In our study, ST191 was the dominant ST during the 7 years of our study and showed stable genomic variations. However, when other STs combined, a tendency of increasing virulence genes was observed without additional changes in antimicrobial resistance of CRAB in restricted hospital environment.

ST191 is a predominant strain isolated in Korea [30] known for expressing $bla_{\text{OXA-23}}$, which is responsible for the high rate of carbapenem resistance. The trait that allows this strain to emerge as a highly successful nosocomial pathogen through the acquisition of various genomes was also manifested in our study. ST191 had relatively large SNP distance, with a median distance of 1291 SNPs between strains. Furthermore, ST191 had

higher insertion sequences than other types, indicative of the frequent recombination and gene rearrangement events. However, no cumulative change in AMR genes and virulence genes was observed over time. Only gene that showed increased prevalence was *strA*. This gene is related to aminoglycoside resistance and frequently found in AbaR-like islands along with other genes such as *tetA*(B), *tetR*(B), *CR2*, *strB*, and orf4b [31]. We observed no simultaneous increase in other AMR genes in this study.

It is worth nothing that heterogenous group of ST were isolated simultaneously. ST208/ST1806 was the sequence type with most abundant AMR genes. This sequence type only contained $bla_{\text{PER-1}}$. $bla_{\text{PER-1}}$ is relatively uncommon gene for *A.baumannii*, but frequently reported as being implicated in various virulence mechanisms [26,32]. This strain was isolated mostly in 2012 in our study and additional spread of this gene was not noted. ST447 which well into IC I was isolated in the latter part of our study period. ST447 possessed fewer AMR genes. As this strain was more frequently isolated from patients with shorter hospital stay, it was introduced from the outside rather than being an indigenous strain. This type was equipped with β-lactam resistance genes without any other resistance genes. As with other strains, ST447 also had $bla_{\text{OXA-23}}$-ΔATPase modules, indicating that it carries Tn*2006*, Tn*2008*, or Tn*2009*. While Tn*2006* was mostly transferred by mobile elements, the spread of Tn*2008* and Tn*2009* was entirely dependent on the clonal dissemination of the bacterial host [30]. In light of ST447 containing all of these transposons, this type might have been under selective antibiotic pressure for a long period of time. It is possible that the previously susceptible strain may gain gene only for carbapenem resistance during the study period, but the widespread use of carbapenems may contribute to the emergence of this type. The density of virulence genes was still low, and this type of strain possessed relatively lower insertion sequences, which are commonly found in other STs. It defies the concern posed by a previous study in Korea that reported the emergence of a new strain that would provoke dissemination of more antibacterial resistance [33].

Increased number of virulence genes over time demands immediate attention. Even though previous studies suggested certain association between antimicrobial resistance and virulence [34], our study demonstrated linear correlation between the two did not exist. It is worth noting that more than half of highly significant virulence genes were possessed by all of these carbapenem resistant strains. A slight increase in the number of virulence genes was reported in the year 2015, probably owing to the emergence of ST451/ST1809. Gene specifically related to functioning of porin (*carO)* [35] had decreased attributing enhanced antimicrobial resistance. However, genes previously considered important in forming biofilm and iron utilization (*bauA, pgaC*) [34,36] were decreased in number.

Increased genes were ACICU_00075-ACICU_00080, ACICU_00086, ACICU_00087, which are presumably associated with immune evasion [37]. Even though further evaluation is warranted regarding authentic effect of these putative virulence genes, gradual accumulation of virulence gene which is transmitted by horizontal transfer emphasise the need for more intensive infection control strategies to prevent re-infections of CRAB.

We failed to observe any significant difference in patient outcomes according to STs. As more than 80% patients died within 28 days of the first isolation of the organism, patients may be in serious conditions and hence died before the analysis reached a conclusive stage. However, previous studies also suggested that phenotypical characteristics are not in accordance with genotypical results. It is possible that individual virulence factors may not be important for *A. baumannii* virulence in human host [38], and the same virulence factor may play a different role in different habitats [39]. The expression of virulence-associated genes could be under different regulation in pathogenic and non-pathogenic species [40].

We found no discrepancies between phenotypic and genotypic results in terms of carbapenem resistance. This observation confirms the results of a previous study, wherein WGS accurately identified resistance to β-lactam antibiotics for gram-negative bacterial pathogens [41]. Whether this observation holds true for other types of antimicrobial agents warrants further studies.

The present study has a few limitations. First, only patients admitted to ICUs were involved, limiting further epidemiological investigation. Second, the relationship between strains, such as evolutionary linkage, was not determined. Third, the detailed comparative genomics to verify novel genes associated with colistin resistance were not analysed.

V. CONCLUSION

In conclusion, this study confirms the absence of accumulation of antimicrobial resistance determinants while the number of virulence genes increased in CRAB. Genetic transfer between subtypes cannot be ruled out.

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ABSTRCT (IN KOREAN)

전체 지놈 염기 서열 분석 방법을 통한 침습적 감염을 일으키는 카바페넴 내성 *A.baumannii*의 유전적 성향 분석 및 이의 임상적 의미

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김민형

Acinetobacter baumannii 는 병원성 감염의 주요 원인 균주이다. 이 연구에서 우리는 2009년 1월부터 2015년 7월 까지 세브란스 병원 중환자실에 입원한 환자의 혈액에서 분리된 카바페넴 내성 *A.baumannii* 분자생물학적 유전 변화를 보고자 하였다. 연구는 전체 98주의 카바페넴 내성 *A.baumannii에 대해* Illumina MiSeq platform 을 이용한 Whole genome sequencing (WGS) 및 multi-locus sequence typing (MLST)을 진행하여 이루어졌다. 항균제 내성 유전자는 ResFinder database 에 염기서열을 대조하여 확인하였고, 병독 인자 유발 유전자는 Large Scale blast score ratio (LS-BSR) 를 이용하여 확인하였다. 역학적 특성은 환자의 병원 입원시 의무 기록을 통해 확인하였다. Oxford scheme 을 이용한 MLST 분석 결과, 분리된 카바페넴 내성 *A.baumannii* 전체 10종류의 sequence type으로 분류됨을 확인하였고, 그 중 ST191이 대다수(n = 59)를 차지함을 확인하였다. 항균제 내성 유전자 중 bla_{OXA-23} 는 대부분의 균주에서 발견되었으며, sequence type 에 따라 항균제 내성 유전자의 조합이 다름을 확인할 수 있었다. 비교적 적은 수의 항균제 내성 유전자를 보유한 ST447 와 ST451/ST1809 이 연구 후반부에 동정되었다. 병독성 유전자는 연구 기간 동안 그 개수에 있어 증가하는 성향을 보였는데, ST191 만을 따로 분리하여 보았을 때에는 변동이 없었다. MLST types, 항균제 내성 유전자 및 병독성 유전자의 구성이 전체 환자의 예후에 큰 영향을 미치지 않는

것으로 확인되었다. 결론적으로, 연구 기간 동안 침습적 감염을 일으키는 카바페넴 내성 *A.baumannii*의 항균제 내성 및 병독성 유전 인자는 연구 기간 동안 큰 변동을 보이지 않는 것으로 확인되었다. 또한, 이 균주의 유전적 성향은 전체적인 환자의 예후에도 영향을 미치지 않음을 확인할 수 있었다.

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