Complete genome sequence of *Acinetobacter baumannii* XH386 (ST208), a multi-drug resistant bacteria isolated from pediatric hospital in China

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ABSTRACT

Acinetobacter baumannii is an important bacterium that emerged as a significant nosocomial pathogen world-wide. The rise of *A. baumannii* was due to its multi-drug resistance (MDR), while it was difficult to treat multi-drug resistant *A. baumannii* with antibiotics, especially in pediatric patients for the therapeutic options with antibiotics were quite limited in pediatric patients. *A. baumannii* ST208 was identified as predominant sequence type of carbapenem resistant *A. baumannii* in the United States and China. As we knew, there was no complete genome sequence reproted for *A. baumannii* ST208, although several whole genome shotgun sequences had been reported. Here, we sequenced the 4087-kilobase (kb) chromosome and 112-kb plasmid of *A. baumannii* XH386 (ST208), which was isolated from a pediatric hospital in China. The genome of *A. baumannii* XH386 contained 3968 protein-coding genes and 94 RNA-only encoding genes. Genomic analysis and Minimum inhibitory concentration assay showed that *A. baumannii* XH386 was multi-drug resistant strain, which showed resistance to most of antibiotics, except for tigecycline. The data may be accessed via the GenBank accession number CP010779 and CP010780.

Specifications

Organism/cell line/tissue	*Acinetobacter baumannii*
Sex	n/a
Sequencer or array type	Hiseq and PacBio
Data format	Analyzed
Experimental factors	Genome sequencing of an antimicrobial resistant strain
Experimental features	The complete genome sequence of a clinical strain of *A. baumannii* was sequenced and annotated to show the multidrug resistant genes.
Consent	n/a
Sample source location	Hangzhou, China

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/nuccore/CP010779
http://www.ncbi.nlm.nih.gov/nuccore/CP010780

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2. Experimental design, materials and methods

2.1. Introduction

Acinetobacter baumannii is an important bacterium which emerged as a significant nosocomial pathogen worldwide [1]. It caused bloodstream infection, pneumonia, endocarditis and so on [2]. The rise of *A. baumannii* was due to its multi-drug resistance, while it was difficult to treat multi-drug resistant *A. baumannii* with antibiotics [3,4]. It caused by *A. baumannii* had a strong potential to develop antimicrobial resistance, which largely related to mobile genetic elements [5].

Carbapenme resistance in *A. baumannii* was mediated most by oxacillinases (OXAs) and less by metallo-β-lactamases (MBLs) [6]. Carbapenem resistance in *A. baumannii* was increasing worldwide, and was considered as a marker of emerging antibiotic resistance [7]. CRAB infection was also a growing problem in the pediatric population. The children were susceptible to infections while the therapeutic options with antibiotics were quite limited. However, the research focusing treatment options on CRAB infections in children was limited. The physicians were forced to use the data extrapolated from the adult literature [8].
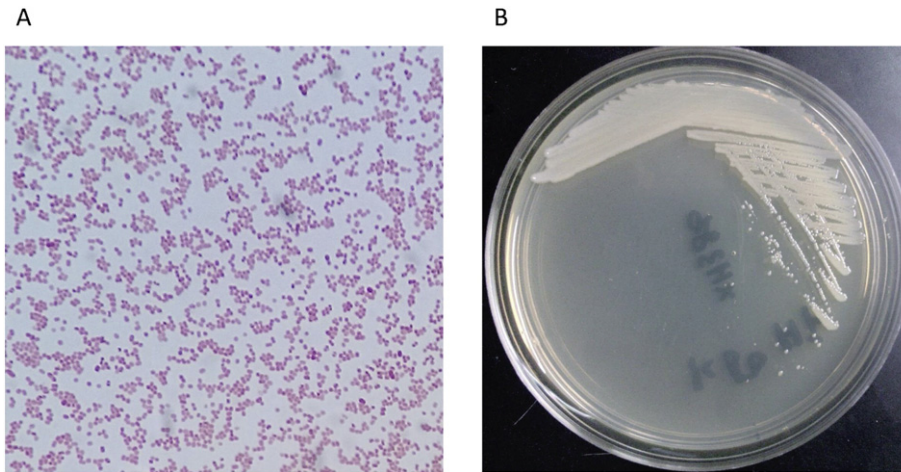
Fig. 1. Cellular and colonial morphology of *A. baumannii* XH386 Gram stained (A) (1000×) and grown on LB agar (B).

For CRAB, sequence types (STs) belonging to the clonal complex 92 (CC92) and the pan-European clonal lineage II (EUII) were predominant in the United States. Of them, *A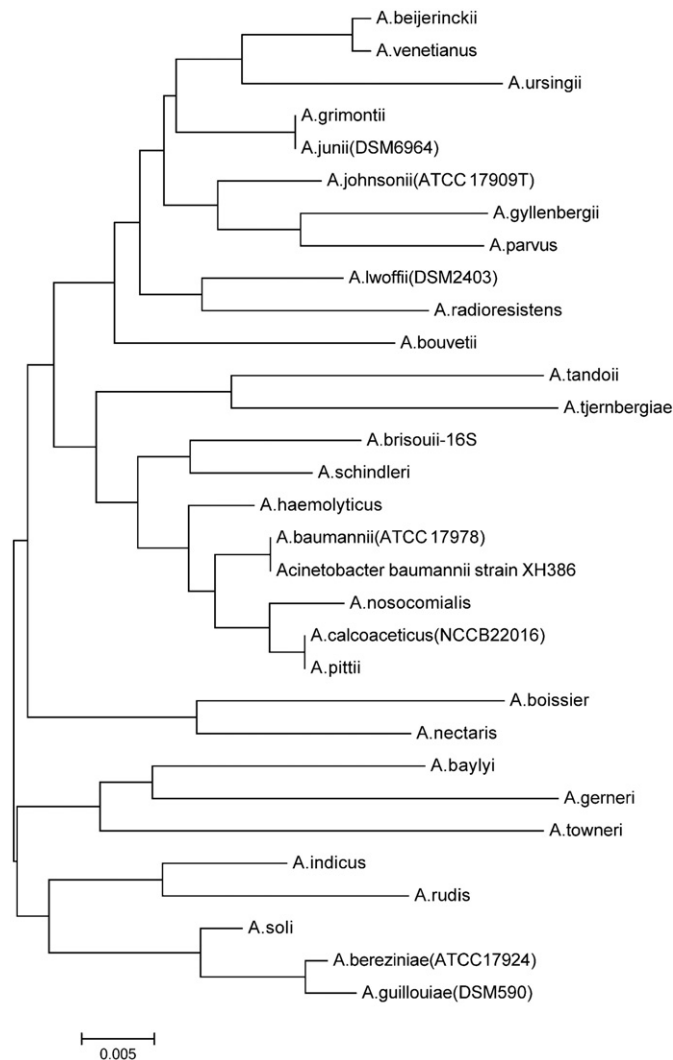. baumannii* ST208 was one of the two most common STs of carbapenem-non-susceptible isolates [9]. Recently, ST 208 had been identified as predominant ST of Carbapenem Resistant *A. baumannii* (CRAB) in China [10,11]. These high prevalence of ST208 carrying bla_{OXA-23} indicated that ST 208 was an emerging lineage mediating the spread of carbapenem resistance via bla_{OXA-23} [10].

The mobility of the resistance genes was mainly mediated by insertions sequences and transposons. The complete genome would be very useful to study the horizontal transferred resistance genes. Most of *A. baumannii* strains that harbored complete genome were isolated from adult patients. *A. baumannii* strain XH386 reported in the paper was isolated from a pediatric patient. This would be helpful to understand whether there was difference between *A. baumannii* strains isolated from adult patient and pediatric patient. As we knew, there was no complete genome sequence of ST208, although several whole genome shotgun sequences had been reported [12]. Here, we present the complete genome sequence of *A. baumannii* XH386 (ST208), which was isolated from a pediatric hospital in China, together with a summary classification and a set of features.

3. Organism information

3.1. Classification and features

A. baumannii XH386 is a non-fermentative, strictly aerobic, non-motile, non-pigmented, catalase-positive and oxidase-negative Gram-negative coccobacilli (Fig. 1). The strain grew on simple microbiological media optimally at ~37 °C, forming smooth colonies of ~2 mm diameter. To evaluate the phylogenomic relationships between *A. baumannii* XH386 and other strain in this genus, Phylogenetic tree was generated with MEGA 6.0 using neighbor-joining method with 500 bootstraps and standard settings. 16S rRNA gene sequences of *Acinetobacter* spp. were derived from NCBI GenBank. The phylogenetic neighborhood of *A. baumannii* XH386 in a 16S rDNA gene sequence based tree was showed in Fig. 2.

To evaluate the phylogenomic relationships between *A. baumannii* XH386 and other strains in this species *A. baumannii*, comparisons between all the strains were calculated as percentages of similarity using Gegennes (version 2.2.1). Then, the percentage of similarity was used

Fig. 2. (A) Phylogenetic tree of *Acinetobacter* spp. 16S rRNA gene sequences were derived from NCBI GenBank. The tree was generated with MEGA 6.0 using Neighbor-Joining method with 500 bootstraps and standard settings.

Table 1
Summary of genome: one chromosome and one plasmid.

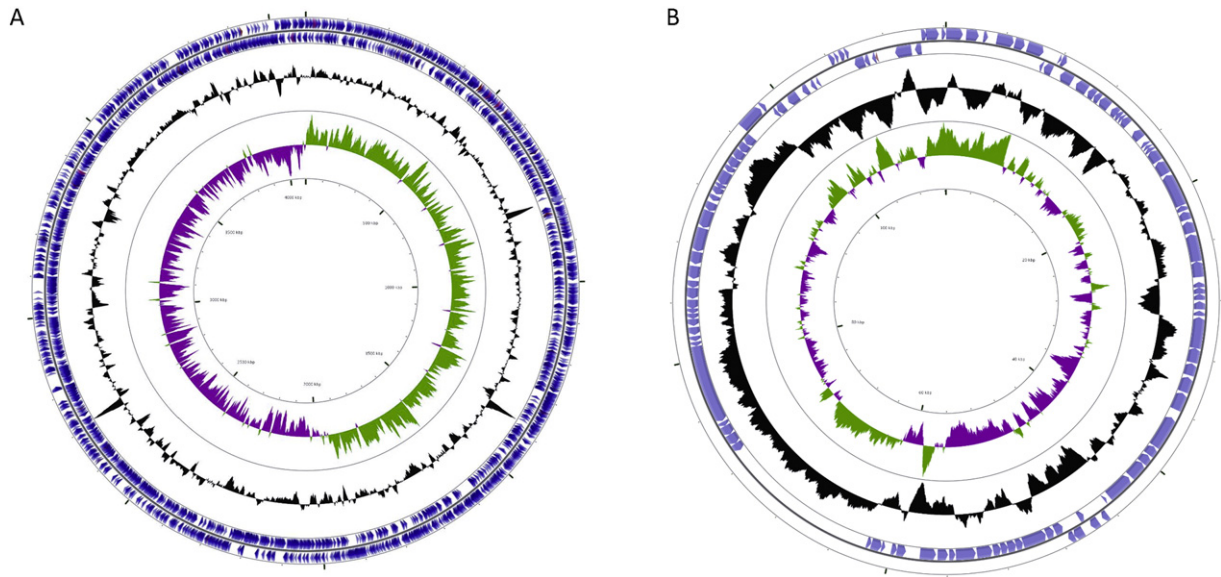| Label	Size (Mb)	Topology	INSDC identifier	RefSeq ID
Chromosome 1	4.08	Circular	PRJNA273343	CP010779.1
Plasmid 1	0.11	Circular	PRJNA273343	CP010780.1

Fig. 3. Graphical map of the chromosome (A) and the plasmid pAB386 (B) of *A. baumannii* XH386. From outside to the centre: Genes on forward strand, genes on reverse strand, GC content (Black), GC skew (purple/olive).

to generate a phylogenomic tree with SplitsTree (version 4.13.1). The phylogenomic relationship in *A. baumannii* was shown in Fig 4A.

4. Genome sequencing information

4.1. Genome project history

The genome was selected based on the isolation site of the strain XH386. *A. baumannii* XH386 was a multi-drug resistant bacteria isolated from a female patient, 10Y3M, with acute bronchopneumonia in a pediatric hospital in Hangzhu, China on May 29, 2014. The genome sequence was completed on 25 Jan., 2015. Annotation was performed by the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP).

4.2. Growth conditions and genomic DNA preparation

A. baumannii XH386 was cultured to mid logarithmic phase in 50 ml of LB medium at 37 °C. DNA for sequencing was extracted via a QIAamp DNA minikit (Qiagen Valencia, CA) followed the protocol of the manufacturer. The quality of DNA was determined by gel electrophoresis

Table 2
Nucleotide content and gene count levels of the genome.

Attribute	Genome (total)	
	Value	% of total[a]
Genome size (bp)	4,087,343	100
DNA coding (bp)	3,627,022	88.7
DNA G + C (bp)	1,596,791	39.1
DNA scaffolds	1	100
Total genes	4062	100
Protein coding genes	3968	97.7
RNA genes	94	2.3
Pseudo genes	26	0.6
Genes in internal clusters	Not determined	Not determined
Genes with function prediction	3887	95.7
Genes assigned to COGs	3039	74.9
Genes assigned Pfam domains	3268	80.4
Genes with signal peptides	322	21.3
Genes with transmembrane helices	864	2.3
CRISPR repeats	2	

[a] The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome. Also includes 26 pseudogenes and 6 frameshifted genes.

and NanoDrop 2000 spectrophotometer (Nano-drop Technologies, Wilmington, DE).

4.3. Genome sequencing and assembly

The genome of *A. baumannii* XH386 was sequenced at Meiji Biotechnology Company (Shanghai, China) using a hybrid of the Illumina and Pacific Biosciences (PacBio) technologies. An Illumina standard shotgun library was constructed, and then was sequenced using the Illumina HiSeq 2000 platform. 3,798,266 reads totaling 953 Mb were generated

Table 3
Number of genes associated with the 25 general COG functional categories.

Code	Value	% of total[a]	Description
J	235	5.79	Translation
A	1	0.02	RNA processing and modification
K	269	6.62	Transcription
L	131	3.23	Replication, recombination and repair
B	0	0.00	Chromatin structure and dynamics
D	39	0.96	Cell cycle control, mitosis and meiosis
Y	0	0.00	Nuclear structure
V	66	1.62	Defense mechanisms
T	117	2.88	Signal transduction mechanisms
M	186	4.58	Cell wall/membrane biogenesis
N	55	1.35	Cell motility
Z	0	0.00	Cytoskeleton
W	3	0.07	Extracellular structures
U	55	1.35	Intracellular trafficking and secretion
O	121	2.98	Posttranslational modification, protein turnover, chaperones
C	201	4.95	Energy production and conversion
G	153	3.77	Carbohydrate transport and metabolism
E	263	6.47	Amino acid transport and metabolism
F	82	2.02	Nucleotide transport and metabolism
H	143	3.52	Coenzyme transport and metabolism
I	221	5.44	Lipid transport and metabolism
P	183	4.51	Inorganic ion transport and metabolism
Q	67	1.65	Secondary metabolites biosynthesis, transport and catabolism
R	238	5.86	General function prediction only
S	210	5.17	Function unknown
-	1023	25.18	Not in COGs

[a] The total is based on the total number of protein coding genes in the annotated genome.
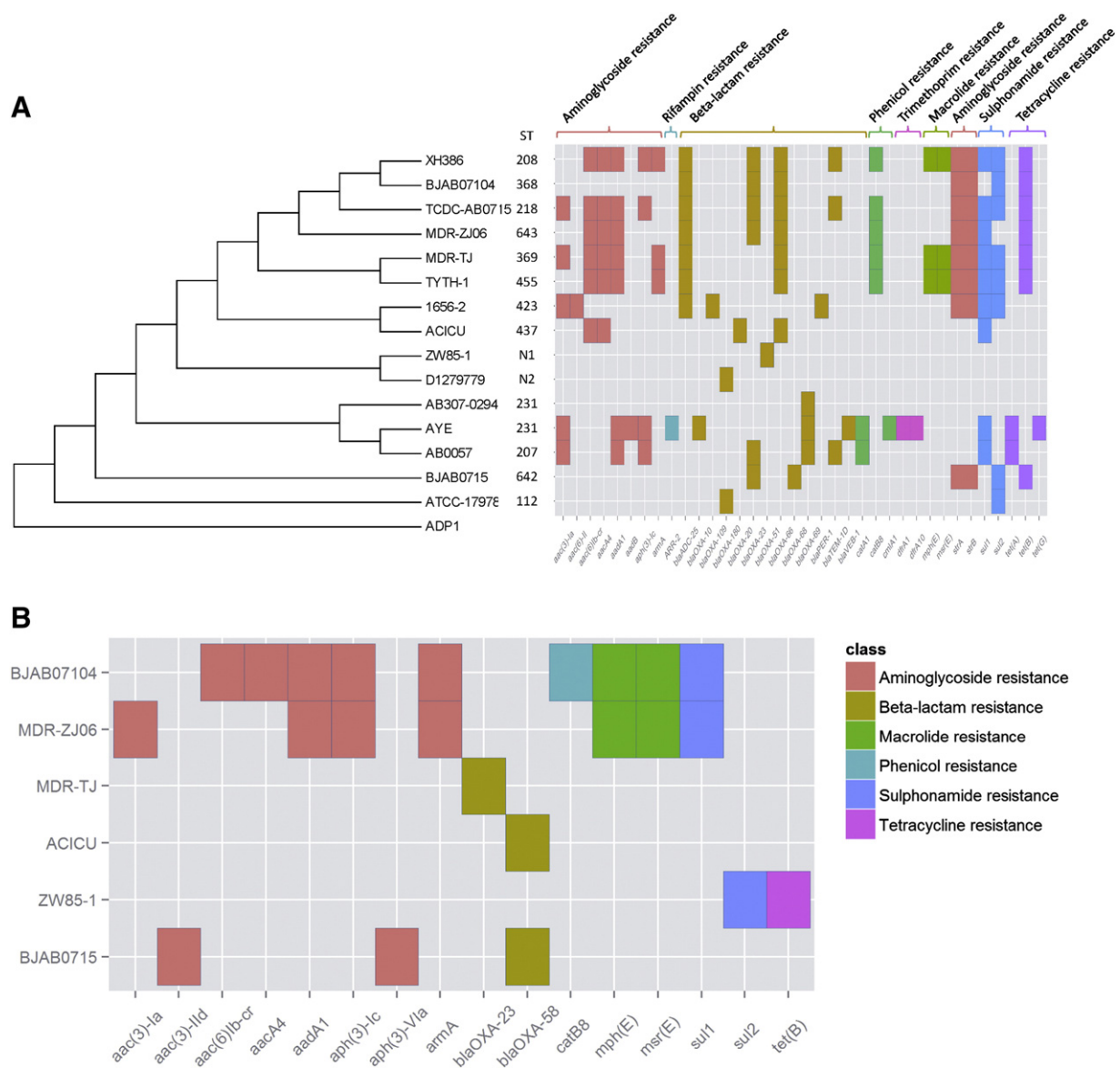
Fig. 4. (A) Phylogenetic tree of phylogenetic tree displaying the relationship between *A. baumannii* and selected strains of the same species. Comparisons between the strains were calculated as percentages of similarity using Gegennes. Then, the percentage of similarity was used to generate a phylogenomic tree with SplitsTree and MEGA. The centre of the figure showed the MLST of these *A. baumannii* strains. The left of the figures showed the abundance of the resistance genes among these *A. baumannii* strains. The resistance genes were detected by ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/), and the heat map of the resistance genes was plotted by the R package "ggplot2". (B) The abundance of the resistance genes among plasmids of these *A. baumannii* strains. The resistance genes were detected by ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/), and the heat map of the resistance genes was plotted by the R package "ggplot2".

from the standard shotgun library. A PacBio SMRTbell™ was constructed and sequenced on the PacBio RS platform. 150,292 raw PacBio reads yielded 76,398 adapter trimmed and quality filtered subreads totalling 355 Mb. *De novo* assembly of the read sequences was performed using continuous long reads following the Hierarchical Genome Assembly Process (HGAP) workflow (PacBio DevNet; Pacific Biosciences) as available in SMRT Analysis v2.3.0, and then Breseq v0.25b with Illumina short reads. The final assembly is based on 953 Mb of Illumina standard PE and 355 Mb of PacBio post filtered data, which provides an average 232× Illumina coverage and 54.76× PacBio coverage of the genome, respectively (Table 1).

4.4. Genome annotation

Annotation of *A. baumannii* XH386 was finished using the NCBI PGAAP annotation pipeline and manually checked. The pipeline uses

Genemark to predict open reading frames (ORF) and searches against Proteins Clusters. Protein coding genes were searched against the NCBI RefSeq database using BLASTp. COG functional categories assignment of the ORFs were archived by BLAST against the COG database. InterPro searches were also done to identify conserved domains in each ORF.

5. Genome properties

The genome of *A. baumannii* XH386 is 4,199,500 nucleotides 39.1% GC content and contain one 4,087,343 bp circular chromosome and one 112,157 bp circular plasmid (Fig. 3). Among of the 4062 genes, predicted 3968 were protein-coding genes, and 94 RNAs; 26 pseudogenes were also identified. The genome summary and distribution of genes into COG functional categories are listed in Tables 2 and 3.

Table 4
Antibiotic resistance profiles of *A. baumannii* XH386.

Antibiotic class	Resistance gene	Predicted phenotype	Accession number
Aminoglycoside	*aph(3′)-Ic*	Aminoglycoside resistance	X62115
	aacA4		M60321
	aadA1	Aminoglycoside resistance	JQ414041
	armA	Aminoglycoside resistance	AY220558
	aph(3′)-Ic	Aminoglycoside resistance	X62115
	strA	Aminoglycoside resistance	M96392
	strB	Aminoglycoside resistance	M96392
Beta-lactam	*blaADC-25*	Beta-lactam resistance	EF016355
	blaOXA-66	Beta-lactam resistance	FJ360530
	blaTEM-1D	Beta-lactam resistance	AF188200
	blaOXA-23	Beta-lactam resistance	HQ700358
Fluoroquinolone	*aac(6′)Ib-cr*	Fluoroquinolone and aminoglycoside resistance	EF636461
MLS — macrolide, lincosamide and streptogramin B	*msr(E)*	Macrolide, Lincosamide and Streptogramin B resistance	EU294228
	mph(E)	Macrolide resistance	EU294228
Phenicol	*catB8*	Phenicol resistance	AF227506
Sulphonamide	*sul1*	Sulphonamide resistance	CP002151
	sul2	Sulphonamide resistance	GQ421466
Tetracycline	*tet(B)*	Tetracycline resistance	AP000342

The abundance of the resistance genes among *A. baumannii* strains XH386 and other strains in this species were detected by ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/). The phylogenetic tree, MLST and resistance genes of *A. baumannii* strains was combined showed in Fig. 4A. The distribution of antibiotic resistance genes in *A. baumannii* XH386 was also shown in Table 4. Fig. 4B showed the distribution of resistance genes in the plasmids harbored by the *A. baumannii* strains. The difference of the distribution of antibiotic resistance genes between chromosome and plasmid demonstrate that the antibiotic resistance genes more often appeared in chromosome. *A. baumannii* XH386 was showed resistance to all antibiotics tested except tigcycline, namely tobramycin, gentamicin, levofloxacin, ciprofloxacin, cefoperazone-sulbactam, amoxicillin–clavulanic acid, piperacillin–tazobactam, ampicillin, ceftriaxone, cefepime, cefoxitin, imipenem, aztreonam, cefazolin, nitrofurantoin, sulfamethoxazole–trimethoprim (Table 5).

6. Insights from the genome sequence

The detection of *bla*OXA-23 explained the resistance to carbapenem. The existence of *aac(6)Ib-cr*, *aacA4*, *aadA1*, *aph(3)-Ic* and *armA* showed good correlation to the resistance of tobramycin and gentamicin. *A. baumannii* XH386 demonstrated more resistance genes than sensitive strains, but not the other resistance ST strains, that indicated the emergence of ST208 had affected by other factors, e.g. show high fitness in clinical environment, more virulence.

7. Conclusions

A. baumannii ST208 was identified predominant ST of Carbapenem Resistant *A. baumannii* in the United States and China. Although several whole genome shotgun sequences of *A. baumannii* ST208 had been reported, there was not complete genome sequence of ST208 so far. In current study, a complete genome of *A. baumannii* ST208 was reported. And the genomic analysis showed that multiple antibiotic resistance genes were detected in the genome, including resistance to aminoglycoside, beta-lactam, fluorequinolone, macrolide, sulphonamide and tetracycline. The genome sequence of *A. baumannii* XH386 would provide deeper insight into the molecular resistance mechanisms and it might facilitate the development of clinical research to control the antibiotic resistance in *A. baumannii*.

Table 5
The susceptibility profile of *A. baumannii* XH386.

Antimicrobial drug	MIC (mg/L)
Tobramycin	>= 128
Gentamicin	>= 16
Levofloxacin	>= 8
Ciprofloxacin	>= 4
Cefoperazone-sulbactam	14
Amoxicillin-clavulanic acid	>= 32
Piperacillin-tazobactam	>= 16
Ampicillin	>= 32
Ceftriaxone	>= 64
Cefepime	>= 64
Cefoxitin	>= 64
Imipenem	>= 16
Aztreonam	>= 64
Cefazolin	>= 64
Nitrofurantoin	>= 512
Sulfamethoxazole-trimethoprim	>= 320
Tigecycline	2

Nucleotide sequence accession number

This complete genome sequence of *A. baumannii* XH386 has been deposited at DDBJ/EMBL/GenBank under the accession number CP010779 and CP010780.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.12.002.

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