

Characterization of a small plasmid carrying the carbapenem resistance gene *bla*_{OXA-72} from community-acquired *Acinetobacter baumannii* sequence type 880 in China

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Background: *Acinetobacter baumannii* has emerged as an important pathogen associated with hospital- and community-acquired infections. Community-acquired *A. baumannii* pneumonia is characterized by a fulminant course and high mortality rates. In this study, we report the identification of a community-acquired carbapenem-resistant *A. baumannii* strain carrying the *bla*_{OXA-72} gene.

Methods: This *A. baumannii* isolate was recovered from a male patient diagnosed with community-acquired pneumonia, septic shock, and respiratory failure. Antimicrobial susceptibility testing were performed and the minimum inhibitory concentrations were determined by the broth microdilution method. Whole-genome sequencing was performed using both long-read MinION and short-read Illumina platforms to fully characterize the *bla*_{OXA-72}-carrying plasmid of the *A. baumannii* A52. The in silico multilocus sequence typing and genomic epidemiological analysis of the closely related isolates were further elucidated by our recently updated BacWGSTdb server.

Results: The isolate was resistant to meropenem and remained susceptible to several other antimicrobial agents. Whole-genome sequencing and bioinformatics analysis indicated that this *A. baumannii* isolate belonged to the rare sporadic clone sequence type 880 and the *bla*_{OXA-72} gene was located on the 8,493-bp plasmid pA52-OXA-72. This plasmid exhibited only partial similarity to different OXA-72-encoding plasmids (size range: 8,771–12,056 bp) in various *Acinetobacter* spp. recovered from patients and other reservoirs in different countries.

Conclusion: This study described the first case of fulminant carbapenem-resistant community-acquired *A. baumannii* pneumonia caused by a rare sporadic clone in China. Adequate surveillance is warranted to monitor the emergence of *A. baumannii* as a community pathogen.

Keywords: *Acinetobacter baumannii*, community-acquired pneumonia, *bla*_{OXA-72}, carbapenem resistance, plasmid

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Introduction

Over the past decades, *Acinetobacter baumannii* has become a common causative agent of multidrug-resistant, hospital-acquired infections worldwide. In addition, it has been shown to be an important cause of community-acquired infection.^{1,2} Unlike hospital-acquired *A. baumannii*, community-acquired pneumonia caused by *A. baumannii* is

characterized by a fulminant course and high mortality rates.³ Carbapenem-resistant *A. baumannii* is a major threat for public health. In 2017, the World Health Organization classified this pathogen as the top priority for the development of additional antibiotics.⁴ The most important mechanism of carbapenem resistance in *A. baumannii* is associated with the production of carbapenem-hydrolyzing class D OXA-type β -lactamases. Of note, five groups of OXA-type carbapenemases (ie, OXA-23-, OXA-24/40-, OXA-51-, OXA-58-, and OXA-143-like) are frequently encountered.⁵ The gene *bla*_{OXA-72} – one of the most important allelic variants within the *bla*_{OXA-24/40} group – was initially identified in an *A. baumannii* strain isolated in 2004 in Thailand (GenBank accession no. AY739646). Subsequently, *bla*_{OXA-72}-carrying *A. baumannii* strains from human and animal origins have been widely identified. However, thus far, few *bla*_{OXA-72}-positive *A. baumannii* strains have been reported in China and the genetic context of *bla*_{OXA-72} is largely unknown.⁶ In the present study, we reported the first identification of a carbapenem-resistant *A. baumannii* isolate associated with fulminant community-acquired pneumonia. This isolate belonged to a rare sporadic clone, harboring both the *bla*_{OXA-72} and the intrinsic *bla*_{OXA-259} genes. Whole-genome sequencing and microbiological analysis were performed to elucidate its mechanism of resistance to carbapenems.

Materials and methods

A 66-year-old male patient was hospitalized with symptoms indicative of community-acquired pneumonia (CAP), septic shock, respiratory failure, and fever. *A. baumannii* isolate A52 was cultured from the sputum sample of the patient within 24 hrs after admission. This patient resided in the countryside, without a history of recent travel or hospitalization. The presence of CAP was considered if pneumonia was not acquired in a hospital, and the interval between the onset of symptoms and previous discharge from hospital was >30 days. Community-associated *A. baumannii* was defined as isolates cultured from sputum and/or a blood specimen obtained from CAP patients and collected within 48 hrs after admission.^{3,7} The quality of the sputum specimen for culture was determined through microscopy. The patient was successfully treated with a one-week administration of imipenem and linked to a good prognosis. The initial identification of species was performed using Vitek 2 (bioMérieux, Marcy-l'Étoile, France) and Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Corp., Billerica, MA, USA). *A. baumannii* A52 was subjected to

antimicrobial susceptibility testing by the microdilution broth method for the following antimicrobial agents: amikacin, ceftazidime, cefotaxime, cefepime, ciprofloxacin, colistin, gentamicin, imipenem, meropenem, minocycline, piperacillin, and tigecycline, which were purchased from Sigma-Aldrich (St. Louis, MO, USA). The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines, except for tigecycline.⁸ Although officially there are no clinical breakpoints for tigecycline against *A. baumannii* given by either CLSI or European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, the US FDA breakpoints (MIC \leq 2 mg/L for susceptibility and MIC \geq 8 mg/L for resistance) or EUCAST breakpoints version 8.0 (MIC \leq 1 mg/L for susceptibility and MIC $>$ 2 mg/L for resistance) for Enterobacteriaceae are commonly applied (http://www.eucast.org/clinical_breakpoints). *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as quality control strains for both species identification and antimicrobial susceptibility testing.

Whole-genome sequencing (WGS) of *A. baumannii* A52 was performed using both the HiSeq X10 (Illumina, San Diego, CA, USA), with the 150 bp paired-end protocol, and the MinION (Nanopore, Oxford, UK) platforms. Hybrid assembly of both short Illumina reads and long Nanopore reads was constructed using Unicycler v 0.4.7 with the Pilon v1.23 option on for the modification of the assembled reads.⁹ The genome annotation was performed using NCBI Prokaryotic Genome Annotation Pipeline. Antibiotic resistance genes was queried using the ResFinder database at the Center for Genomic Epidemiology (<http://www.genomicepidemiology.org/>).¹⁰ *In silico* multilocus sequence typing (MLST) analysis and bacterial source tracking for implementing both single-nucleotide polymorphism (SNP) and core genome multilocus sequence typing (cgMLST) strategies were performed by our recently updated BacWGSTdb server.¹¹ Easyfig was used to analyze the genetic surroundings of antimicrobial resistance genes.¹² The nucleotide sequence of the chromosome and plasmids of *A. baumannii* isolate A52 have been deposited in NCBI GenBank under accession numbers CP034092-CP034097. The sputum sample and clinical isolate of *A. baumannii* isolate A52 were generated as part of routine hospital laboratory procedures. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Sir Run Run Shaw Hospital, Zhejiang University School of

Medicine, China. Written informed consent was obtained from the patient, which included the publication of the case details.

Results and discussion

Antimicrobial susceptibility testing showed that *A. baumannii* A52 was resistant to meropenem. However, it remained susceptible to several other antimicrobial agents, including imipenem (Table S1). In particular, for the broth microdilution method, the minimum inhibitory concentrations of meropenem and imipenem were 8 mg/L and 2 mg/L, respectively. In addition to carbapenemases, the permeability defects, such as over-expression of efflux system, may also contribute to carbapenem resistance in *A. baumannii*. The sequences of efflux pump regulatory genes were analyzed, which are known to be involved in antibiotic resistance, namely, the AdeR/S two-component system and AdeN and AdeB, which regulate the AdeIJK and AdeAB efflux pumps, respectively. The regulatory efflux pumps AdeR and AdeS of *A. baumannii* A52 showed 99% (1 substitution) and 100% amino acid sequence identities, respectively, with those of *A. baumannii* ATCC 17978, whereas AdeN and AdeB of *A. baumannii* A52 showed 100% amino acid sequence identities with those of *A. baumannii* ATCC 17978. These findings highlight the efflux pump regulatory genes in this strain may have a negative influence in resistance to carbapenems. WGS to assess the sequence type (ST) identified *A. baumannii* A52 as ST880 and ST77, according to the Oxford and Pasteur MLST schemes, respectively. It contained three genes

(*bla*_{OXA-72}, *bla*_{OXA-259}, and *bla*_{ADC-26}) conferring resistance to β -lactams, which were not preceded by an insertion element. This finding was consistent with the phenotypic data. In addition, we found that this strain lacked the *A. baumannii* antibiotic resistance island, which confers resistance to multiple antibiotics (eg, aminoglycosides, beta-lactams, sulfonamides, and tetracyclines). The absence of an antibiotic resistance island in *A. baumannii* may explain the high susceptibility of the community-acquired strain to antibiotics.

The *bla*_{OXA-72} gene was located on the 8,493-bp plasmid pA52-OXA-72. The backbone of the pA52-OXA-72 plasmid is almost identical to that of pAbIHIT32296 – a *bla*_{OXA-72}-carrying plasmid in *A. baumannii* recovered from a captive grey parrot in Germany. The shared sequence of these plasmids contains the *bla*_{OXA-72} gene. However, in pA52-OXA-72, this gene is in a different location and orientation. Furthermore, the plasmid pA52-OXA-72 exhibited only partial similarity to other OXA-72-encoding plasmids (size range: 8,771–12,056 bp) in various *Acinetobacter* spp. recovered from patients in different countries (Figure 1, Table S2). Plasmid pA52-OXA-72 contained 11 open reading frames and encoded a replication protein belonging to the replication protein 3 superfamily.¹³ In addition, it carried genes encoding a putative type II toxin–antitoxin system (VapC2-VapB2). These genes were also present in other OXA-72-encoding plasmids (eg, pIEC338SCOX) and may be involved in plasmid maintenance (Figure 2).¹⁴ The *bla*_{OXA-72} gene was flanked by XerC/XerD recombination sites, which were

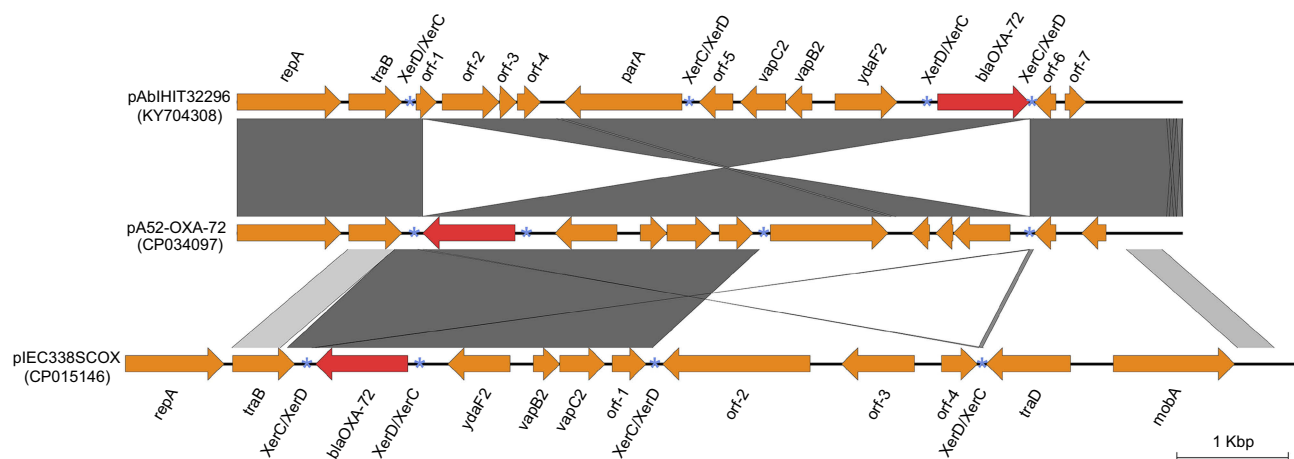


Figure 1 Comparison of the homologous regions shared by pA52-OXA-72, pAbIHIT32296, and pIEC338SCOX. Open arrows represent coding sequences (red arrows, *bla*_{OXA-72}) and indicate the direction of transcription. The arrow size is proportional to the gene length. *repA*, replicase gene; *traB*, gene coding bacterial conjugation Trb1-like family protein; *ydaF2*, gene encoding putative ribosomal N-acetyltransferase; *vapB2*, gene encoding putative antitoxin; *vapC2*, gene encoding putative ribonuclease/toxin; *parA*, gene coding plasmid partition protein A; XerC/XerD, specific site of the tyrosine recombinases XerC and XerD (indicated by asterisks).

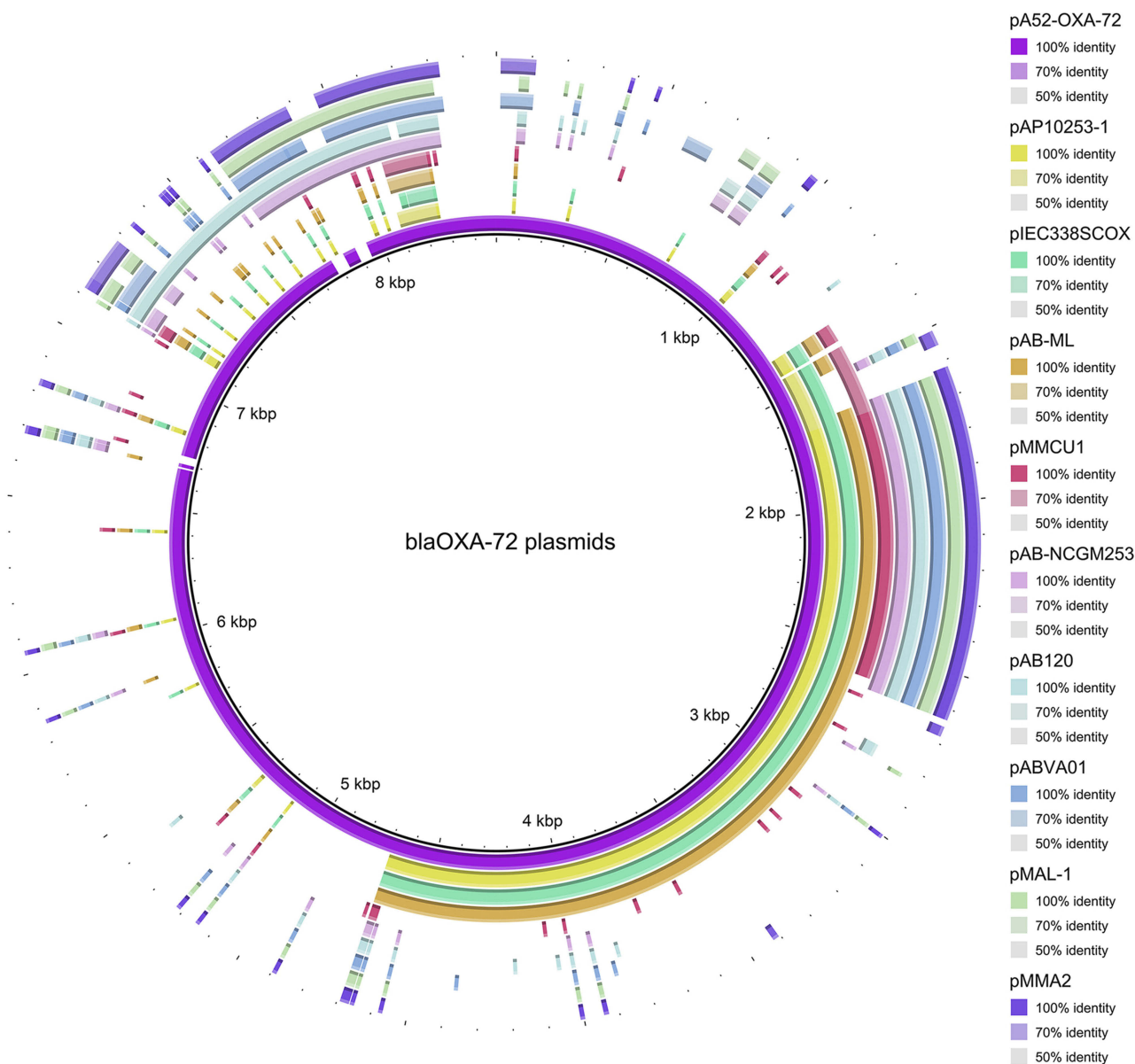


Figure 2 Plasmid sequence alignment of *bla*_{OXA-72}-carrying plasmids that revealed partial sequence identity to pA52-OXA-72.

identical to those of previously reported plasmids. This finding indicated the importance of mobilization through the site-specific recombination mechanism. As previously suggested, the XerC/XerD-sites may be involved in a site-specific recombination system responsible for the mobilization of the *bla*_{OXA-72} gene among *Acinetobacter* spp. Moreover, a conserved region (ie, *ydaF2-vapB2-vapC2-orf1*) in pA52-OXA-72 also bracketed with the XerC/XerD-sites and showed high sequence identity to the regions in plasmid pIEC338SCOX. This observation suggests that different plasmids may exchange carbapenem-hydrolyzing class

D OXA-type β -lactamases genes and other components within a restricted region between the two closest XerC/XerD binding sites.

The origin of *A. baumannii* A52 remains unknown. We analyzed the phylogenetic relationship between *A. baumannii* A52 and a total of 3,417 *A. baumannii* strains currently deposited in the NCBI GenBank database. This analysis was performed using the following two bacterial source tracking strategies: SNP and cgMLST. Both strategies suggested that *A. baumannii* A52 belongs to a rare sporadic clone. Notably, the closest relative was an ST1325 (single-locus variant of ST880) isolate known

as HC9436, which was recovered from the endobronchial intubation tube of a patient in Honduras in 2015 (Table S3, Figure S1). However, the present patient had no history of recent overseas travel or direct contact with foreigners. Hence, there was no epidemiological link identified between this Chinese patient and the previously reported case. *A. baumannii* CAP exhibits seasonal variation, with a higher incidence reported in the warmer and more humid months of the year.³ Consistent with previously reported cases, the present patient was admitted in August (ie, the hot and rainy season in China).^{15–17}

The OXA-72-producing *A. baumannii* isolates have been mainly obtained from hospitals and other reservoirs (ie, livestock/companion animals, and environment) in South America, Southern Asia, and Eastern Europe.^{18–20} More recently, a cross-sectional study reported a high prevalence and clonal dissemination of OXA-72-producing *A. baumannii* in a Chinese hospital, corresponding to the predominant international clone 2. However, noninternational clone 2 *A. baumannii* strains (ie, ST78 in Germany, ST1 in Serbia, and ST79 in Brazil) carrying *bla*_{OXA-72} have also been reported.^{20–22} Therefore, based on the genetic flexibility mediated by the elements for site-specific recombination, the *bla*_{OXA-72}-carrying plasmids of *A. baumannii* may represent powerful vehicles for the acquisition, horizontal-transfer, and evolution of carbapenem resistance.

Conclusion

We reported the identification of a carbapenem-resistant community-acquired *A. baumannii* isolate in China. This isolate belongs to a rare sporadic clone harboring a unique *bla*_{OXA-72}-carrying plasmid. Our study highlights the transmission potential of OXA-72-producing *A. baumannii* in the community. Continuous surveillance is necessary to monitor the transmission dynamics of the *bla*_{OXA-72} gene in the community.

Abbreviation list

A. baumannii, *Acinetobacter baumannii*; CAP, community-acquired pneumonia; MIC, minimum inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; WGS, whole-genome sequencing; MLST, multilocus sequence typing; ST, sequence type; cgMLST, core genome multilocus sequence typing; SNP, single nucleotide polymorphism.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

ZR and YXX conceived and designed the study. HQJ and QYS collected samples and performed experiments, ZR and HQJ performed data analysis, ZR wrote the paper. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

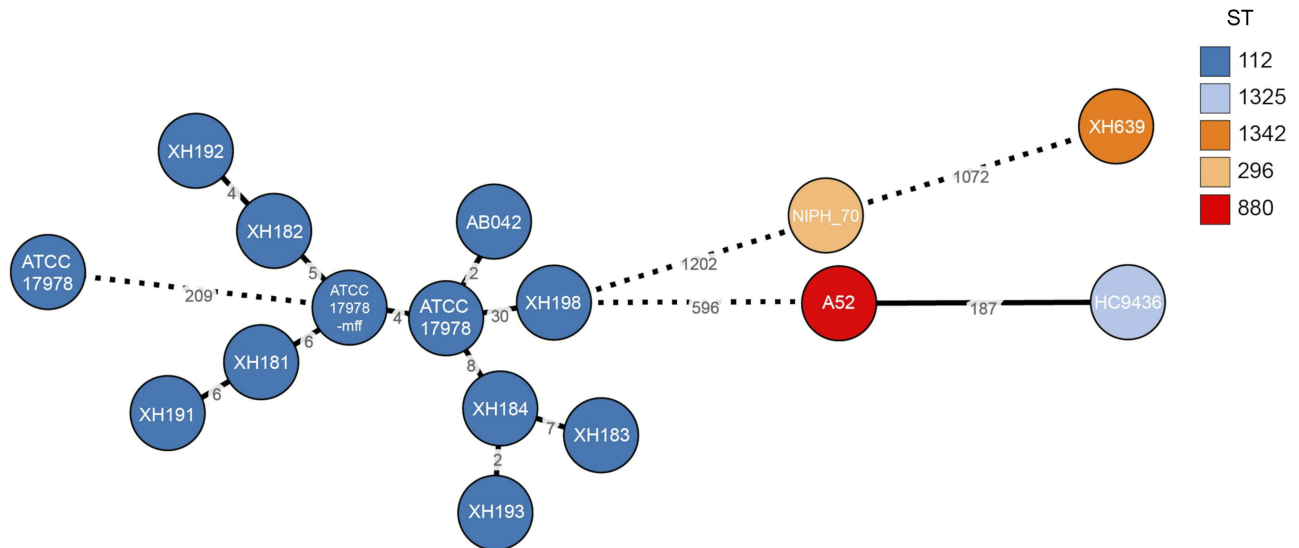


Figure S1 Phylogenetic relationship between *A. baumannii* A52 and the closely related *A. baumannii* strains currently deposited in the NCBI GenBank database. The lines connecting the circles indicate the clonal relationship between different isolates and the digital numbers on the lines illustrate the number of allelic differences.

Table S1 Antibiotic resistance profile of *A. baumannii* isolate A52

Antimicrobial	MIC (mg/L)	Interpretation
amikacin	0.5	S
cefepime	4	S
cefotaxime	8	S
ceftazidime	4	S
ciprofloxacin	0.125	S
colistin	0.125	S
gentamicin	1	S
imipenem	2	S
meropenem	8	R
minocycline	0.5	S
piperacillin	16	S
tigecycline*	0.5	S

Notes: Antimicrobial susceptibilities, reported as MIC (mg/L), were interpreted in accordance with established breakpoints: amikacin (S≤16, I=32, R≥64), cefepime (S≤8, I=16, R≥32), cefotaxime (S≤8, I=16-32, R≥64), ceftazidime (S≤8, I=16, R≥32), ciprofloxacin (S≤1, I=2, R≥4), colistin (S≤2, R≥4), gentamicin (S≤4, I=8, R≥16), imipenem (S≤2, I=4, R≥8), meropenem (S≤2, I=4, R≥8), minocycline (S≤4, I=8, R≥16), piperacillin (S≤16, I=32-64, R≥128), tigecycline-FDA (S≤2, I=4, R≥8), tigecycline-EUCAST (S≤1, I=2, R>2). *The EUCAST breakpoints for Enterobacteriaceae are applied to interpret the MIC of tigecycline against *A. baumannii*.

Table S2 Characterisation of *Acinetobacter* spp. plasmids containing *bla*_{OXA-72} gene recovered from different countries

Plasmids	Length (bp)	Host	Collection Year	Isolation source	Country	Accession number	References
pA52-OXA-72	8,493	Human	2015	Sputum	China	CP034097.1	This study
pAbIHIT32296	8,493	Grey parrot	2016	Nose	Germany	KY704308.1	1
pIEC338SCOX	10,498	Human	2014	Tracheal secretion	Brazil	CP015146.1	2
pAPI0253-I	10,498	Human	2012	-	Brazil	KY499579.1	2
pAB-ML	12,056	Human	2012	-	Taiwan	KT022421.1	3
pMCMCU1*	8,771	Human	-	Blood	Spain	GQ342610.1	4
pAB-NCGM 253	8,970	Human	2012	-	Japan	AB823544.1	5
pABI20	10,879	Human	2010	Respiratory tract	Lithuania	JX069966.1	6
pABVA01*	8,963	Human	2000	Bronchoalveolar lavage fluid	Italy	FM210331.1	7
pMAL-I	9,810	Human	-	Urine	Serbia	KX230793.1	8
pMMA2*	10,679	Human	-	Blood	Spain	GQ377752.1	4

Notes: *These plasmids contain *bla*_{OXA-24}, a single-nucleotide variant of *bla*_{OXA-72}.

Table S3 Information of closely related strains to *A. baumannii* A52

Isolate	Accession number	ST	Host	Disease	Isolation Source	Country State	Collection Year	Different alleles
HC9436	NQXM01	1325	Homo sapiens	Acinetobacter infection	Endobronchial tube	Honduras: Cortes, San Pedro Sula	2015-09-15	187
XH198	MDWM01	112	-	-	-	China: Hangzhou	2014-03	596
ATCC_17978	CP018664	112	Homo sapiens	Nosocomial infection	Blood	-	-	608
ATCC_17978-mff	CP012004	112	Homo sapiens	Meningitis	-	Canada	2014	608
AB042	CP019034	112	Homo sapiens	Nosocomial infection	Lab mutation of ATCC 17978	-	-	610
XH181	MDWH01	112	-	-	-	China: Hangzhou	2014-03	610
XH182	MDWJ01	112	-	-	-	China: Hangzhou	2014-03	610
XH184	MDWL01	112	-	-	-	China: Hangzhou	2014-03	613
XH192	MDWI01	112	-	-	-	China: Hangzhou	2014-03	613
XH183	MDWK01	112	-	-	-	China: Hangzhou	2014-03	614
XH193	MDWF01	112	-	-	-	China: Hangzhou	2014-03	614
XH191	MDWG01	112	-	-	-	China: Hangzhou	2014-03	615
ATCC_17978	CP000521	112	-	-	-	-	-	763
NIPH_70	APRC01	296	Homo sapiens	-	Tracheal secretion	Czech Republic: Praha	1992	1229
XH639	LYKQ01	1342	Homo sapiens	-	Sputum	China: Hangzhou	2014-08-29	1340

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