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OXA-23 Is a Prevalent Mechanism Contributing to Sulbactam Resistance in Diverse *Acinetobacter baumannii* Clinical Strains

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Acinetobacter baumannii is one of the most important and threatening pathogens for health care-associated infections (HAI), and the treatments for multidrug-resistant *A. baumannii* (MDRAB) are limited (1, 2). Sulbactam serves as an alternative and effective option to combat these infections because of its intrinsic antimicrobial activity against the *Acinetobacter* genus (2). Unfortunately, increasing resistance to sulbactam compound in *A. baumannii* has been reported during the last decade (3, 4). Previous studies have demonstrated that β-lactamases such as TEM-1 and ADC confer sulbactam resistance in *A. baumannii*; however, they did not fully explain the sulbactam resistance phenomenon in each clinical strain (5–7). Therefore, we screened all β-lactamase genes in clinical isolates by whole-genome sequencing and aimed to assess the role of other β-lactamases apart from TEM-1 and ADC in sulbactam resistance of *A. baumannii*.

Eighty-eight clinical isolates, including 57 sulbactam-nonsusceptible isolates and 31 sulbactam-susceptible isolates, were selected from a broad geographic distribution across China (7). The presence of the β-lactamase genes $bla_{\text{TEM-1}}$, IS*Aba1*-bla_{ADC}, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-58}}$, $bla_{\text{PER-1}}$, and $bla_{\text{CARB-2}}$ were screened by whole-genome sequencing (Fig. 1a). Of note, in the sulbactam-nonsusceptible group, 54 (94.74%) strains possessed the $bla_{\text{OXA-23}}$ gene, while in the sulbactam-susceptible group, only 4 (12.90%) isolates were positive for the $bla_{\text{OXA-23}}$ gene ($P < 0.001$). These results indicated that $bla_{\text{OXA-23}}$ might facilitate sulbactam resistance in *A. baumannii*.

To further elucidate the correlation between $bla_{\text{OXA-23}}$ and sulbactam resistance, deletion and complementation of this gene were performed. See Table S1 in the supplemental material for a list of the strains and plasmids used in this study. The $bla_{\text{OXA-23}}$ gene was deleted in a clinical *A. baumannii* isolate, A2265, which carried one copy of $bla_{\text{OXA-23}}$ without $bla_{\text{TEM-1}}$ or IS*Aba1*-bla_{ADC}. The strain A2265 Δ$bla_{\text{OXA-23}}$ was complemented with the recombinant plasmid pYMAb2-Hyg^r::IS*Aba1*-$bla_{\text{OXA-23}}$. The changes in the MICs were determined (Table 1). Notably, A2265 Δ$bla_{\text{OXA-23}}$ exhibited increased susceptibility to sulbactam (8-fold) compared to that of A2265, and a similar trend was observed in the MICs of cefoperazone-sulbactam, imipenem, and meropenem, but no obvious change in MIC values was found in other agents. The MICs of A2265 Δ$bla_{\text{OXA-23}}$ (pYMAb2-Hyg^r::IS*Aba1*-$bla_{\text{OXA-23}}$) for sulbactam, cefoperazone-sulbactam, imipenem, and meropenem returned to levels at or above those of strain A2265.

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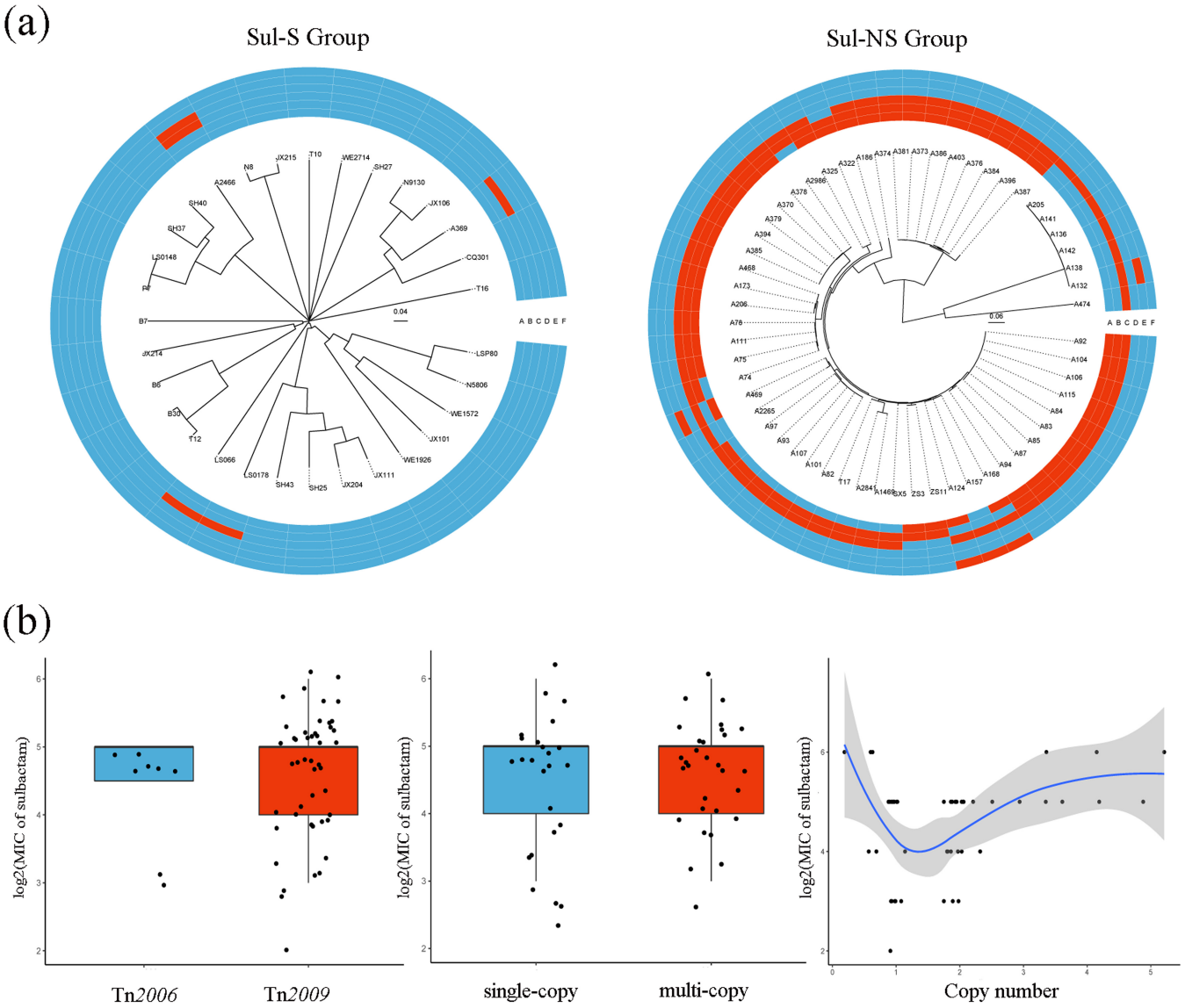

FIG 1 (a) Heatmap of the presence of the β-lactamase genes of all selected clinical *A. baumannii* strains. Due to the lack of a breakpoint for sulbactam alone, we chose ≤4 mg/liter as the temporary susceptibility breakpoint on the basis of the CLSI susceptibility breakpoint for ampicillin-sulbactam (≤8/4 mg/liter) in *A. baumannii*. On the basis of the breakpoint, all clinical isolates were divided into sulbactam-nonsusceptible or sulbactam-susceptible groups. Sul-S group, sulbactam-susceptible group; Sul-NS group, sulbactam-nonsusceptible group; A, bla_{TEM-1}; B, IS*Aba1*-bla_{ADC}; C, bla_{OXA-23}; D, bla_{OXA-58}; E, bla_{PER-1}; F, bla_{CARB-2}. Red color represents positive, and the blue color represents negative. (b) Correlation of the characteristics of the bla_{OXA-23} gene associated with sulbactam MIC. All the bla_{OXA-23}-positive strains were divided into two groups according to the transposase types carrying bla_{OXA-23} or copy number of bla_{OXA-23}: Tn*2009* and Tn*2006*, as well as single-copy group (copy number <1.5) and multicopy group (copy number >1.5).

Among the 58 bla_{OXA-23}-positive strains, 47 isolates were correlated with Tn*2009*, 8 isolates were associated with Tn*2006*, and the remaining 3 isolates in the sulbactam-susceptible group were found to carry only a partial sequence of bla_{OXA-23}. Tn*2007* and Tn*2008* were not detected. No significant difference in the sulbactam MICs (log2) of the Tn*2009*-group and Tn*2006*-group was found ($P > 0.05$) (Fig. 1b). The copy numbers of all bla_{OXA-23}-positive strains were measured by quantitative real-time PCR. A total of 25 isolates were found to carry a single copy of bla_{OXA-23}, while 30 isolates had at least two copies of bla_{OXA-23}. The sulbactam MICs of the multicopy group were similar to that of the single-copy group ($P > 0.05$) (Fig. 1b). Furthermore, the MIC of sulbactam did not correlate with the copy number of bla_{OXA-23} ($r = 0.263$) (Fig. 1b).

OXA-23 is notoriously not inhibited by sulbactam, but sulbactam is also a well-known agent used directly against the *Acinetobacter* genus as an inhibitor. Although

TABLE 1 MICs of the knockouts and the transformants

Isolate[a]	MIC (mg/liter)[b]											
	SUL	CPS	IPM	MEM	CTX	CAZ	CIP	MIN	GEN	CST	TGC	ATM
A2265	8	16	32	32	16	2	>32	4	>256	0.25	1	12
A2265 Δbla_{OXA-23}	1	1.5	1	1	8	4	>32	2	>256	0.25	1	12
A2265 Δbla_{OXA-23}(pYMAb2-Hyg^r)	1	0.75	1	1	4	6	>32	8	>256	0.25	1	6
A2265 Δbla_{OXA-23}(pYMAb2-Hyg^r::IS*Aba1*-bla_{OXA-23})	64	64	128	128	>32	3	>32	4	>256	0.25	1	24

[a]A2265 Δbla_{OXA-23}, the clinical strain A2265 without the bla_{OXA-23} gene; A2265 Δbla_{OXA-23}(pYMAb2-Hyg^r), the strain A2265 was transformed with a plasmid pYMAb2-Hyg^r; A2265 Δbla_{OXA-23}(pYMAb2-Hyg^r::IS*Aba1*-bla_{OXA-23}), the strain A2265 Δbla_{OXA-23} was complemented with the recombinant plasmid pYMAb2-Hyg^r::IS*Aba1*-bla_{OXA-23}.

[b]SUL, sulbactam; CPS, cefoperazone-sulbactam; IPM, imipenem; MEM, meropenem; CTX, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; MIN, minocycline; GEN, gentamicin; CST, colistin; TGC, tigecycline; ATM, aztreonam.

OXA-23 manifests considerable hydrolytic activity on a variety of β-lactam antibiotics, its effect on sulbactam has not been fully established (8, 9). Our research described the effect of OXA-23 on the sulbactam MIC in a clinical *A. baumannii* strain by gene deletion and complementation experiments. Nevertheless, we found that the sulbactam MIC did not relate to the transposon type or copy number of bla_{OXA-23}. Similar results were found in studies concerning bla_{OXA-23} associated with carbapenem resistance (10, 11). The unstable copy number due to the plasmid location of bla_{OXA-23} mentioned in prior research may be one of the factors (11). Another possible explanation may be the coexistence of other mechanisms, such as bla_{TEM-1}, IS*Aba1*-bla_{ADC}, mutations of penicillin-binding proteins, and overexpression of the efflux pump, which can affect the sulbactam MIC simultaneously (12).

Altogether, the above-described findings suggest that sulbactam resistance in *A. baumannii* is multifactorial and can indeed be affected via OXA-23.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01676-18.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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We declare no conflict of interest.

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