

Letter to the Editor

Dissemination and evolution of bla_{OXA-23} gene among carbapenem-resistant *Acinetobacter baumannii* isolates from central ChinaXiaobing Guo¹, Zaiqiu Cao¹, Zhijian Zhang²¹ Department of Clinical laboratory, First Affiliated Hospital of Zhengzhou University, Henan, China² Basic Medical College, Zhengzhou University, Henan, China**Key words:** *Acinetobacter baumannii*; OXA-23; novel variants; evolutionary study; MLST.*J Infect Dev Ctries* 2016; 10(4):445-448. doi:10.3855/jidc.7498

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Dear Editor,

Recently, increasing resistance to carbapenems in *Acinetobacter baumannii* has emerged rapidly, which severely restricts the clinical treatment for this pathogen [1]. A prevalent beta-lactam resistance mechanism comprises class D beta-lactamase enzymes [2], and clonal outbreaks of carbapenem resistant and OXA-23-producing *A. baumannii* have been reported in many countries [2-4]. However, despite the well-defined evolutionary trajectories of *A. baumannii* [2,5], it remains unclear how bla_{OXA-23} evolves in related strains or in a limited area. Therefore, we isolated 52 carbapenem-resistant *A. baumannii* in central China, and investigated evolutionary paths of bla_{OXA-23} genes.

Fifty-two non-repetitive carbapenem-resistant *A. baumannii* were recovered in Henan province. This current study has been approved by the Ethics Committee of Zhengzhou University. Antimicrobial susceptibility testing was performed by the disk diffusion method, and results were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) 2014 guidelines. The 52 isolates were distributed among eight clinical units, with the majority in ICU (64.5 %). In general, all of the *A. baumannii* isolates were resistant to nine commonly used antibiotics, including Cefoperazone/sulbactam, Piperacillin/tazobactam, Cefotaxime, Ceftazidime, Ciprofloxacin, Ceftriaxone, Cefepime, Imipenem, and Meropenem. Interestingly, on the contrary >50% (31/52) of the *A. baumannii* isolates displayed AMK susceptible.

Genomic DNA was extracted and screened for the presence of bla_{OXA-23}-like, bla_{OXA-51}-like, bla_{OXA-24}-like,

bla_{OXA-58}-like and bla_{OXA-143}-like genes. The bla_{OXA-51} genes, which were hypothesized as intrinsic genes of *A. baumannii* [2,5,6], were detected in all strains. Simultaneously, bla_{OXA-23}-like genes were also found in all 52 strains, and three novel variants were found and assigned as OXA-481, OXA-482 and OXA-483 by the β-lactamase database site [7]. However, bla_{OXA-58}-like or bla_{OXA-143}-like genes were not detected in any of the strains, and only one strain carried bla_{OXA-24} gene. This suggested that OXA-51 and OXA-23 might be causes of carbapenem resistance mechanisms in *A. baumannii* in central China.

Multilocus Sequence Typing (MLST) was performed with primers according to previous studies [8]. All isolated strains seemed to be clonally related; only 4 strains were detected to be ST75, and >90% of these strains belonged to ST92. Interestingly, no mutation was found in ST75. A nationwide research described that the widest distribution of clone CC92 is the principal reason for the rapid increase of carbapenem resistant rate in China [8]. Interestingly, ST92 and ST75 also belonged to CC92, which implies that successful clonal lineage CC92 may be a mechanism associated with carbapenem resistance in central China. The distribution of clonally related strains may result from the simultaneous diversification of multiple lineages with rapid population expansion following a bottleneck [9,10], which is hypothesized to be a consequence of a narrow ecological niche of *A. baumannii*. In other words, the clonally related *A. baumannii* in central China might result from the wide usage of carbapenem antibiotics.

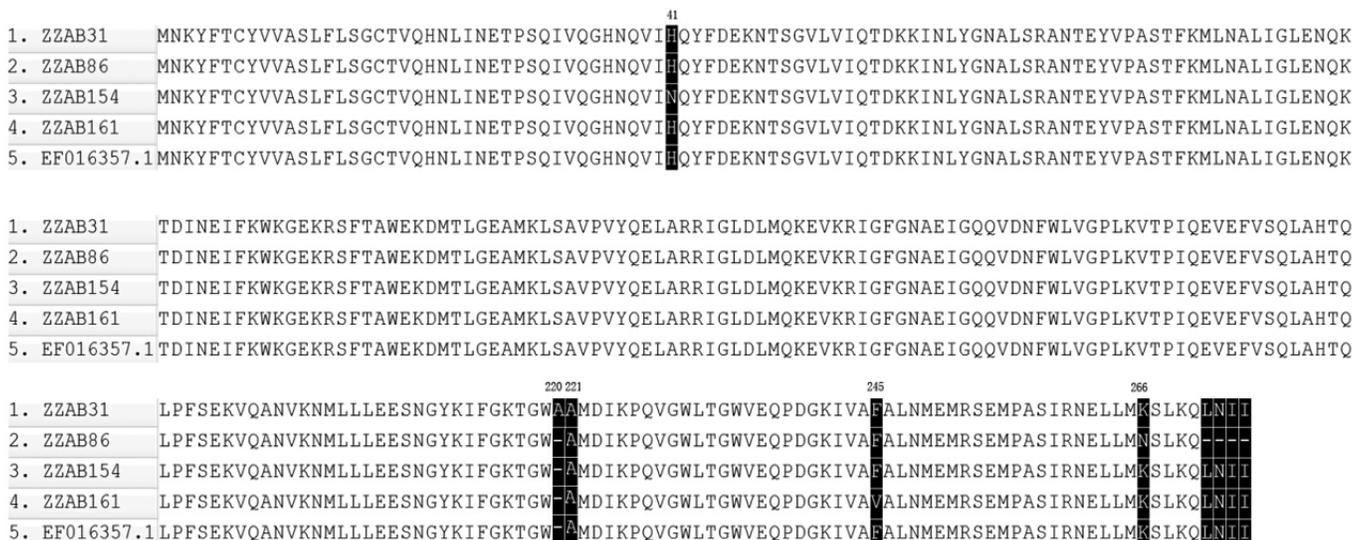
The amino acid sequences of OXA-23 were aligned using CLUSTALW as implemented in MEGA 6 [11]. As shown in the alignment results (Figure 1), four mutations of OXA-23 were detected. BLAST searches indicated that three of them were novel variants (ZZAB86, ZZAB154 and ZZAB161 assigned as OXA-481, OXA-482 and OXA-483, respectively) and the other (ZZAB31) was 100% identical to OXA-146. All of these four strains were isolated from sputum in four different clinical units, and they were resistant to ten commonly used antibiotics. According to previous studies, strains carrying OXA-146 or OXA-49 possess a duplication of A220, adding one residue in the β5-β6 loop and affect the hydrolytic activity toward antibiotics tremendously [12,13]. How other mutations (K266N, H41N and F245V) affect carbapenem resistance is not clear: they may affect kinetics, but mutations do not appear to directly influence the active site; further studies will be carried on *in silico* and *in vitro*.

Using aligned sequences, phylogenetic analysis was conducted in neighbour-joining method, and bootstrap values were obtained after 1,000 replicates. According to the phylogenetic tree of OXA-23 (Figure 2), sequences were divided into several clusters. ZZAB31, OXA-49 and OXA-146 were distributed into one cluster, since they all had the same added residue (A220). Positive selection using bla_{OXA-23} sequences files was identified by Hyphy selection test [11], but no specific position was positively selected.

Variants of OXA-23 were compared in a referential tertiary structure (PDB no.4K0X) by PyMOL Software (PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC). The comparison of OXA-23 variants had indicated that some mutations occurred more frequently than others. For instance, both OXA-146 and OXA-49 have mutated in the β5-β6 loop. Until now, whether the prevalence of bla_{OXA-23} gene results from a previously established multidrug-resistant clonal lineage or whether the success of certain clonal lineages is due to bla_{OXA-23} gene that they acquired is not clear. [2,10] Although the alanine insertion results in 20-fold and 100-fold decreases in K_m against cefotaxime and ceftriaxone respectively [12], isolates carrying bla_{OXA-146} or bla_{OXA-49} only occurred in three different cities across China, which indicates that the evolved antibiotic-resistant genes may not cause a serious prevalence status. The incidence of sign epistasis [14], compensatory mutations [15] as well as co-occurrence mutations [16] may cause the failure of a novel mutation to pass on in the evolutionary trajectory of bla_{OXA-23}.

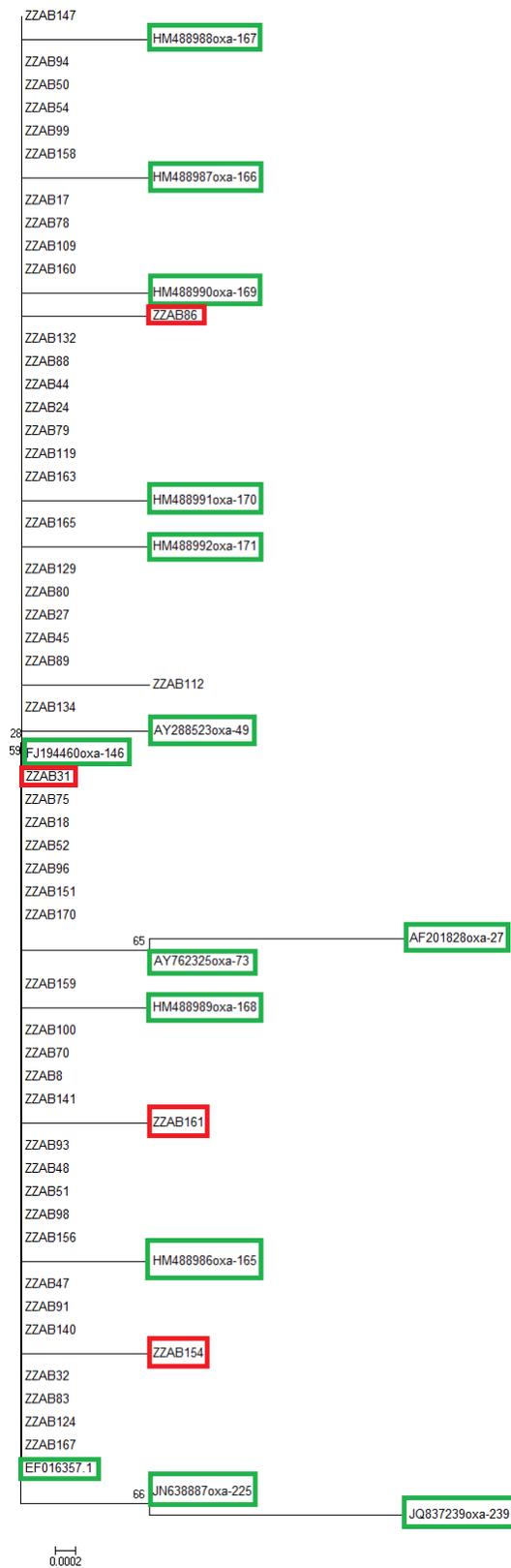
In conclusion, clinical *A. baumannii* strains are clonally related in central China and ST92 may be the dominant strain greatly contributing to the prevalence of bla_{OXA-23}. Although no sufficient results are available to reconstruct the evolutionary path of bla_{OXA-23}, bioinformatic analysis indicates that the loop between β5 and β6 strands of bla_{OXA-23} may evolve under antibiotic therapies.

Figure 1. Alignment of OXA-23-like β-lactamases.



K266N, H41N, F245V and the duplication of A220 (numbered above, shown in black background) are the positions that differ between the five sequences.

Figure 2. Phylogenetic analyses of 66 *A. baumannii* strains.



The accession numbers of the downloaded sequences are squared in green color whilst novel OXA-23 variants are in red in the phylogenetic tree. Bootstrap values obtained after 1.000 replicates are given at the nodes.

Nucleotide sequence accession number

The nucleotide sequence data detected in this work has been deposited in the GenBank database under accession no. KP264122, KP264123, KP264124 and KP264125 (representing ZZAB31, OXA-481, OXA-482 and OXA-483, respectively).

The nucleotide sequences of bla_{OXA-23}-like genes used for evolutionary studies are as follows: bla_{OXA-27}, AF201828; bla_{OXA-49}, AY288523; bla_{OXA-73}, AY762325; bla_{OXA-134}, HQ122933; bla_{OXA-146}, FJ194460; bla_{OXA-165}, HM488986; bla_{OXA-166}, HM488987; bla_{OXA-167}, HM488988; bla_{OXA-168}, HM488989; bla_{OXA-169}, HM488990; bla_{OXA-170}, HM488991; bla_{OXA-171}, HM488992; bla_{OXA-225}, JN638887; bla_{OXA-239}, JQ837239.

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Corresponding author

Xiaobing Guo

Department of Clinical laboratory, First affiliated Hospital of Zhengzhou University, 1 jianshe road, Zhengzhou, Henan, China

Phone: +00-86-13629846026

Email: zzu_guo@126.com

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