Detection of Carbapenemase-producing Genes in the *Acinetobacter baumannii* Causing Infections Isolated in Vietnam by Using the Whole-genome Sequencing Technology

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BACKGROUND

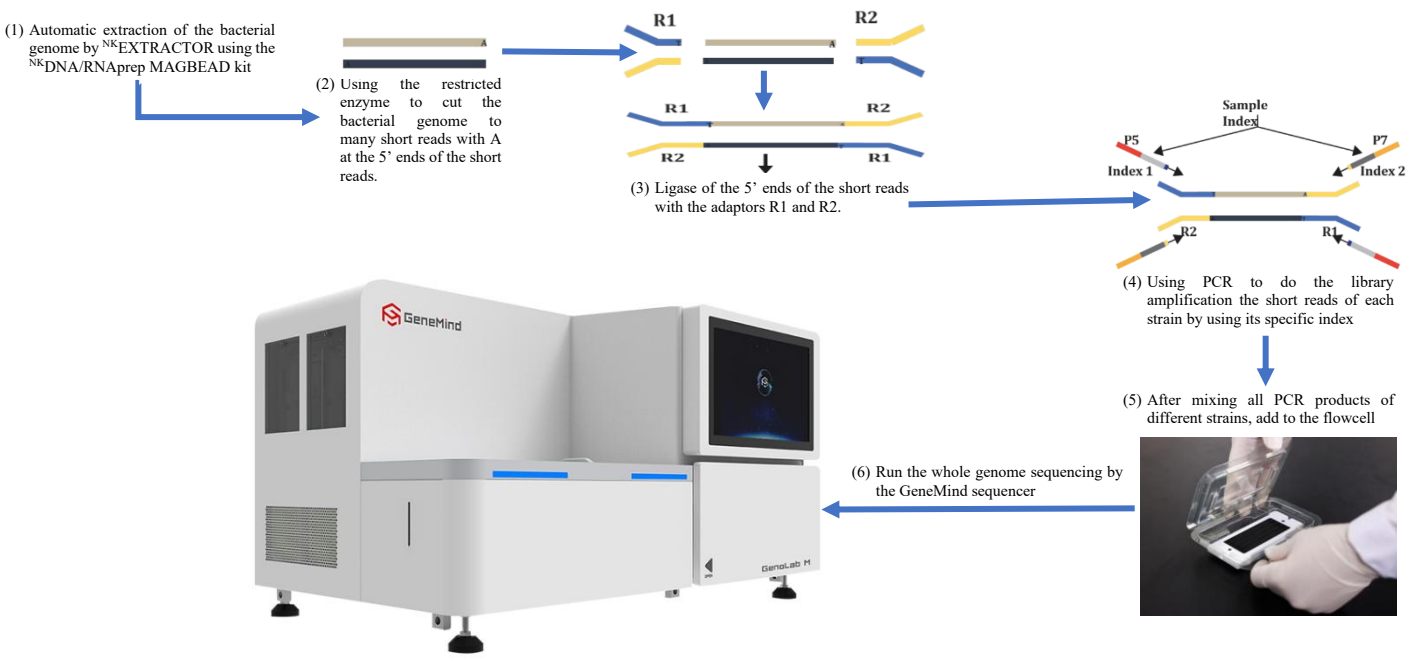
A. baumannii is a challenging pathogen due to its relatively high resistance rate to carbapenem antibiotics. Several different mechanisms contribute to *A. baumannii*'s resistance to carbapenems, primarily through the production of carbapenemase[1,2]. Therefore, understanding the carbapenemase genes of *A. baumannii* is essential.

AIMS OF THE STUDY

To apply whole-genome sequencing technology to infectious *A. baumannii* strains isolated from hospitalized patients in Vietnam to detect carbapenemase-producing genes present in carbapenem-resistant bacterial strains.

MATERIALS AND METHODS

The 301 *A. baumannii* strains studied were recovered and purified, then lysed to recover all DNA, which was fragmented into segments no longer than 300 bp by using restricted enzymes. Next, the adapters were attached to both ends of these DNA fragments using ligase enzymes, followed by PCR amplification for attachment of the index sequences to both ends of the amplified DNA segments. Finally, whole-genome sequencing was performed using a GeneMind device from Shenzhen GeneMind Biosciences Co., Ltd. After sequencing, the obtained data were analyzed using many open-source software to detect the presence of bacterial carbapenemase-producing genes.



Picture 1: The different steps using GeneMind instrument for the whole genome sequencing of the multiple *Acinetobacter baumannii* bacteria isolated from patients hospitalized by infection in Viet Nam

RESULTS

The results showed that all 228 meropenem-resistant *A. baumannii* strains had detectable carbapenemase-producing genes, including OXA23 (86.84%), OXA66 (77.19%), TEM-1 (67.98%); followed by NDM (14.47%), IMP-14 (2.63%), OXA500 (1.75%), OXA72 (0.88%); and the lowest were SHV-11, OXA417, OXA1045, OXA94, OXA217 and OXA317 with only 0.44% presence. In addition to genes producing carbapenemase that destroy carbapenems, the results also detected the A515V mutation in the ftsI gene at a very high rate (71.05%), which prevents carbapenems from binding to PBP3 to exert their effects. The different combinations of the carbapenem resistant genes were also detected with the highest ratio were ftsI_A515V+OXA23+OXA66+TEM-1 (61.40%); followed by ftsI_A515V+OXA23+OXA66 (7.89%), OXA23 (7.46%), NDM (6.14%), OXA23+OXA66+TEM-1 (5.70%), NDM+IMP (2.19%), and NDM+OXA23 (2.19%). then NDM+OXA500 (0.88%), ftsI_A515V+NDM+OXA23+OXA66 (0.88%); and the lowest ratio were NDM+IMP+OXA417 (0.44%), NDM+OXA1045 (0.44%), NDM+OXA94 (0.44%), NDM+SHV11 (0.44%), ftsI_A515V+NDM+OXA23+OXA500+OXA66+TEM1 (0.44%), ftsI_A515V+OXA66+TEM-1 (0.44%), OXA23+OXA72 (0.44%), OXA23+OXA66 (0.44%), OXA72+OXA317 (0.44%), OXA217 (0.44%), OXA930 (0.44%), and OXA500 (0.44%). These carbapenem resistant genes were not detected among 73 trains *A. baumannii* not meropenem resistant.

DISCUSSIONS AND CONCLUSION

Whole-genome sequencing technology has shown that 100% of carbapenem-resistant *A. baumannii* strains possess the carbapenemase-producing gene. This demonstrates that the mechanism of carbapenem resistance in the *A. baumannii* strains causing infections isolated in Vietnam is due to the bacteria producing carbapenemase that destroys carbapenem. The discovery of the genes and genotypes that enable *A. baumannii* to produce carbapenemase in this study provides essential data for testing sensitivity and specificity, as well as for developing technologies for rapid detection of carbapenemase-producing genes such as real-time PCR.

Table 1: The ratio (%) of the different combinations of the carbapenem resistant genes detected in the Imipenem resistant *A. baumannii*. These genes were not detected among *A. baumannii* sensible to imipenem

Gene	%
ftsI_A515V-OXA23-OXA66-TEM-1	61.40%
ftsI_A515V-OXA23-OXA66	7.89%
OXA23	7.46%
NDM	6.14%
OXA23-OXA66-TEM-1	5.70%
NDM-IMP	2.19%
NDM-OXA23	2.19%
NDM-OXA500	0.88%
ftsI_A515V-NDM-OXA23-OXA66	0.88%
NDM-IMP-OXA417	0.44%
NDM-OXA1045	0.44%
NDM-OXA94	0.44%
NDM-SHV11	0.44%
ftsI_A515V-NDM-OXA23-OXA500-OXA66-TEM1	0.44%
ftsI_A515V-OXA66-TEM-1	0.44%
OXA23-OXA72	0.44%
OXA23-OXA66	0.44%
OXA72-OXA317	0.44%
OXA217	0.44%
OXA930	0.44%
OXA500	0.44%

KEY WORDS

Carbapenem reisistant genes, *Acinetobacter baumannii*, NDM, IMP, OXA, ftsI_A515V

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