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Letter to Editor

Microbiology Section

Comparison of 16S rRNA Sequencing and VITEK 2 Analysis for the Identification of *Acinetobacter baumannii* Clinical Isolates: A Study from Southwestern Province of Saudi Arabia

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Dear Editor

Acinetobacter baumannii (A. baumannii) is one of the major causes of nosocomial infections in the hospital environment. Even the World Health Organisation (WHO) has designated *A. baumannii* as a priority pathogen that poses a significant health risk [1]. *A. baumannii* rapidly develops resistance to antimicrobials, and multidrug-resistant strains have been reported in the literature. As a multidrug-resistant and invasive pathogen, it has been identified as an opportunistic pathogen that causes severe infections such as wound infections, pneumonia, meningitis, septicaemia, and urinary tract infections, resulting in high mortality and morbidity rates. Carbapenem-resistant *A. baumannii* is a major global public health threat and imposes a greater burden worldwide, including in Saudi Arabia [2].

Due to the close genetic relationship of some Acinetobacter species, it is difficult to distinguish them phenotypically using standard laboratory techniques. Several genotypic methods are used for the identification of bacterial species, with 16S ribosomal Ribonucleic Acid (rRNA) gene sequencing being one of the most commonly utilised methods for identifying bacterial isolates. Accurate bacterial and species identification is crucial for effective treatment of *A. baumannii* infections [2]. However, to date, there have been no reports comparing 16S rRNA sequencing and VITEK 2 analysis for the identification of *Acinetobacter baumannii* clinical isolates from the Southwestern Province of Saudi Arabia.

Therefore, the present study aimed to compare the phenotypic identification system (VITEK 2) with 16S rRNA sequencing for the identification of *A. baumannii* hospital isolates. A total of 29 clinical isolates of *A. baumannii* were collected from a tertiary hospital in the Southwestern Province of Saudi Arabia and identified using the VITEK 2 system (bioMérieux, Marcy l'Etoile, France). Ethical approval (2016/50A) was obtained from King Fahd Central Hospital, and informed consent was obtained from the participants. All 29 strains were further subjected to identification based on 16S rRNA gene sequencing. The 16S rRNA gene sequencing was performed at Macrogen, Inc. (South Korea), using universal primer pairs (785F 5'(GGA TTA GAT ACC CTG GTA) 3' and 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3') and confirmation primers (27F 5'(AGA GTT TGA TCM TGG CTC AG) 3' and 1492R 5'(TAC GGY TAC CTT GTT ACG ACT T) 3') for *A. baumannii*.

In the present study, the VITEK 2 system correctly identified 93.10% of clinical isolates of *A. baumannii* when compared with the 16S rRNA gene sequencing method [Table/Fig-1]. In contrast,

Strain number	Bacteria identified by 16S rRNA sequencing							Bacteria identification by VITEK 2 system		
	Subject				identities					
	Length in base pair (bp)	Start in bp	End in bp	Coverage in percentage	Match/total in bp	%	Name of bacteria identified	Name of the bacteria identified	Correct (T)/Miss identification (F)	% Correct identification
1	1529	17	1481	95	1464/1465	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
2	1529	17	1481	95	1464/1465	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
3	1529	17	1493	96	1473/1477	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
4	1546	21	1497	95	1473/1479	99	*Stenotrophomonas rhizophilia*	*Acinetobacter baumannii*	F	
5	1529	17	1479	95	1462/1464	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
6	1529	15	1481	95	1466/1467	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
7	1515	1	1473	97	1469/1473	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
8	1529	15	1483	96	1468/1469	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
9	1529	15	1483	96	1468/1469	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
10	1529	17	1493	96	1473/1477	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	93.10
11	1529	15	1480	95	1465/1466	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
12	1529	15	1480	95	1465/1466	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
13	1529	18	1481	95	1463/1464	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
14	1529	15	1481	95	1466/1468	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
15	1529	18	1493	96	1471/1477	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
16	1497	1	1476	98	1473/1476	99	*Stenotrophomonas pavanii*	*Acinetobacter baumannii*	F	
17	1529	18	1481	95	1463/1464	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
18	1529	15	1488	96	1472/1475	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
19	1529	19	1478	95	1459/1460	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	

20	1529	15	1481	95	1466/1467	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
21	1515	1	1461	96	1460/1461	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
22	1529	17	1480	95	1463/1464	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
23	1529	18	1481	95	1463/1464	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
24	1529	17	1493	96	1473/1477	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
25	1529	19	1493	96	1472/1475	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
26	1529	16	1480	95	1463/1465	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
27	1529	17	1478	95	1461/1462	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
28	1529	15	1481	95	1466/1467	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	
29	1529	18	1488	96	1469/1471	99	*Acinetobacter baumannii*	*Acinetobacter baumannii*	T	

[Table/Fig-1]: Comparison of identification of bacterial species by using 16S rRNA sequencing and VITEK 2 system.

Lee MJ et al., reported an accuracy rate of 76.6% for the VITEK 2 system in identifying *A. baumannii* clinical isolates [3]. Additionally, other reports have shown that the VITEK 2 system correctly identified 87.5% of other bacterial clinical isolates compared with 16S rRNA gene sequencing analysis [4]. The VITEK 2 system identifies bacterial isolates based on metabolic activities and/or morphological features. Misidentification of bacterial isolates in the VITEK 2 system can occur due to various reasons: (i) aged culture isolates may not display the expected biochemical characteristics; (ii) different strains of the same species may not express a specific characteristic; (iii) the same strain may produce different outcomes in subsequent testing; (iv) long-term antibiotic therapy may cause isolates from a host to change their typical metabolic features; (v) phenotypic variation can affect the accuracy of species-level identification by automated phenotypic systems; (vi) the databases only contain information on a few species; and (vii) phenotypic systems frequently propose two or more labels with similar probabilities [4]. Therefore, 16S rRNA sequencing may be useful for the correct identification of *A. baumannii* clinical isolates when compared with the VITEK 2 system of identification.

Authors' contributions: VKB conceived the study, contributed to the design, and drafted the manuscript. AA, NK, and MUA assisted with sample collection and provided overall support for the study. AAZH was responsible for collecting the samples. All authors have read and approved the final manuscript.

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