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Breaking The Barrier In Infection Control: A Novel Antigenic Protein Of *Acinetobacter baumannii* For Rapid Point-of-Care Diagnostics

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ABSTRACT

Acinetobacter baumannii detection at an early stage is primarily important for the effective treatment and control of its nosocomial infections. Diagnosis of *A. baumannii* nosocomial infections take some days (5 - 7 days) to produce results in Hospital laboratories by cultures and automated techniques. This delay contributes to high mortality rate associated to *A. baumannii*. Rapid, accurate and cost-effective detection test that will reduce morbidity and mortality associated with delay the. The SAPs of *A. baumannii* was profiled using SDS-PAGE, antigenicity of the profiled SAPs was determined by Western Blot techniques and LC-MS was used to identify novel antigenic biomarker SAPs. Result of SDS-PAGE profiles revealed the presence of 22 SAP bands, Western blot analysis of the profiled SAPs showed (86.4%) of the SAPs from SDS-PAGE profiles were antigenic. IgG and IgM detected a higher percentage (84.2%) each and IgA (57.9%). Three (3) novel antigenic SAPs (40.9, 36.0, and 34.4 kDa) from *A. baumannii*, were identified by Liquid Chromatography Mass Spectrophotometry as OmpA, Omp38, an elongation factor protein and ribosome-recycling factor protein respectively. Identification of these novel antigenic proteins may contribute to the development of rapid diagnostic test for the early detection of *Acinetobacter baumannii* infection in the future.

Keywords: *Acinetobacter baumannii*; Surface-Associated Proteins; Biomarkers, rapid diagnostic test, LC MS, Infectious Control

INTRODUCTION

Acinetobacter baumannii appeared to be top most nosocomial pathogen troubling mostly patients with weakened host defense system in intensive care units and is accountable for several hospital outbreaks of bacterial nosocomial infections (Luo *et al.*, 2020) Elsner, *et al* 2000; Nafarieh *et al.*, 2017; LópezDurán *et al.*, 2020). Nosocomial infections (NIs) caused by *Acinetobacter* are among the main challenges of the health care system globally, particularly in the Hospital ICUs, accident and emergency units, and long-term care homes (Haque *et al.*, 2018). Across the globe, about 1.7 million hospitalized patients yearly acquire NIs, and one (1) in every seventeen (17) patients is lost to NIs (Haque *et al.*, 2018). *Acinetobacter baumannii* is the most frequently encountered isolate in nosocomial infection.

The time taken (5 to 7 days) by the culture and automated methods in the diagnosis of *Acinetobacter* nosocomial infections is an increasing concern due to the increase mortality rate associated with the delay (). It was estimated that *Acinetobacter* BSI are associated with high mortality rates (17-52%), and the time taken to produce laboratory diagnosis result contributed to the hike in mortality rate (Cisneros *et al*, 1996; Wispling hoff *et al*, 2000; Zhou *et al.*, 2019). These emphasize the importance of early diagnosis of the *Acinetobacter* infection to enable effective therapy and management of the disease (Vrancianu *et al.*, 2020, Song *et al.*, 2025).

Substantial number of *Acinetobacter baumannii* surface proteins antigenicity studies focused on OMPs, whereas the SAPs have attracted less attention (Rahbar *et.*, al 2012; Girija, *et al.*, 2021) hence, less is known regarding its SAPs antigenicity.

Serologic tests demonstrated good option for the rapid diagnosis of many bacterial diseases (Busson *et al.*, 2013). Currently, the focus has shifted towards proteins as diagnostic candidate, chiefly targeting bacterial surface proteins, because they are more exposed and thus, more accessible to antibodies. Similar to other gram negative bacteria (Geisinger *et al.*, 2020), *Acinetobacter baumannii* contains lipopolysaccharide (LPS) on the surface of its outer membrane that has been successfully used as antigenic markers for *Salmonella* Typhi for the development of sero diagnostic tests (Ismail, 2000; Ismail, *et al*; 2011; Muralinath *et al.*, 2011; Zahid, *et al*; 2025). This present study explores the surface-Associated proteins of *Acinetobacterbaumannii* with the aim to identify potential proteomic biomarkers for the rapid detection of *Acinetobacterbaumannii* for the development of rapid diagnostic test.

MATERIALS AND METHODS

2.1. Bacteria

Acinetobacter baumannii strains used in this study were obtained from a collection of *A. baumannii* isolates from cases of nosocomial infections in Federal Teaching Hospital and Sir Yahaya Memorial Hospital Birnin Kebbi. *Acinetobacter baumannii* isolation and identification was done by automated technique (ViTEK-2 system Biomerica). The identity of the bacterium was confirmed to a species level by Polymerase Chain Reaction (PCR) amplification of *Acinetobacter rpoB* gene. *Acinetobacter baumannii* ATCC 900 reference strain used was purchased from Belgium Culture Collection Center.

RESULTS

Profiles of *A. Baumannii* ATCC 19606, Reference Strain and *A. Baumannii* AB1001 Clinical Isolate Expressed at 37°C

The SAPs of *A. baumannii* ATCC 19606 reference strain and *A. baumannii* clinical isolates were profiled using 10% SDS-PAGE (Fig.1). Comparisons between the SAPs profiles of reference strain and that of clinical isolates revealed 22 SAP bands. Twenty (20) SAPs bands were present in the reference strain isolate, and twenty-two (22) SAP bands were present in the clinical isolates. The additional two SAP bands seen in clinical isolates are 70.3 kDa and 18.0 kDa. The reference strain shared 20 SAP band (104.0, 96.7, 85.2, 66.2, 62.0, 48.7, 46.0, 45.0, 44.0, 40.9, 36.0, 34.4, 32.0, 30.0, 26.0, 25.0, 23.0, 21.0, 16.0 and 14.0 kDa) with clinical isolates (Table 4.2).

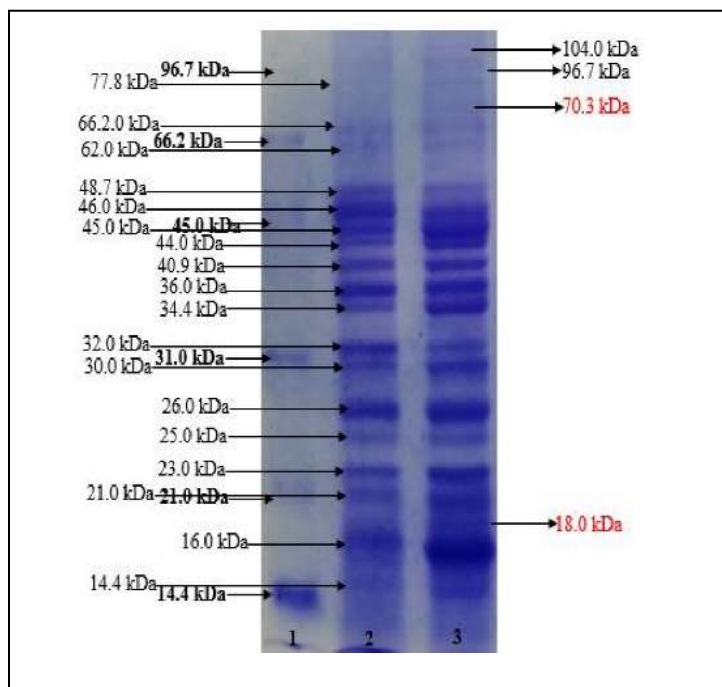


Figure 1: SDS-PAGE SAPs profiles of *A. baumannii* ATCC 19606 reference strain and *A. baumannii* AB1001 clinical isolate


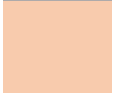
Key:

- Lane1: Protein molecular weight marker
- Lane 2: SAPs of *A. baumannii* ATCC 19606 reference strain
- Lane 3: SAPs of *A. baumannii* AB1001 clinical isolate

Table: 1 Comparative analysis of SAPs profiles of *A. baumannii* ATCC 19606 reference strain and *A. baumannii* AB1001 clinical isolate expressed at 37°C

Number of SAPs bands	Molecular weight of SAPs	<i>A. baumannii</i> ATCC 19606	<i>A. baumannii</i> AB1001
1	104.0 kDa	√	√
2	96.7 kDa	√	√
3	85.2 kDa	√	√
4	70.3 kDa	x	√
5	66.2 kDa	√	√
6	62.0 kDa	√	√
7	48.7 kDa	√	√
8	46.0 kDa	√	√
9	45.0 kDa	√	√
10	44.0 kDa	√	√
11	40.9 kDa	√	√
12	36.0 kDa	√	√
13	34.4 kDa	√	√
14	32.0 kDa	√	√
15	30.0 kDa	√	√
16	26.0 kDa	√	√
17	25.0 kDa	√	√
18	23.0 kDa	√	√
19	21.0 kDa	√	√
20	18.0 kDa	x	√
21	16.0 kDa	√	√
22	14.0 kDa	√	√
TOTAL		20	22

Key:

- √ = Indicate the present of SAP band
- x = Indicate the absence of SAP band
-  SAPs exclusive to *A. baumannii* AB1001
-  SAPs common to *A. baumannii* ATCC & AB1001

Antigenicity study of *A. baumannii* SAPs

Summary of *A. baumannii* antigenic SAPs detected by IgG, IgM, and IgA

Table1: Showing the summary of overall antigenic SAPs detected in *A. baumannii* by the IgG, IgM, and IgA of patient sera positive for *A. baumannii* infection. Out of the 22 *A. baumannii* SAP bands seen in SDS-PAGE, the IgG and IgM detected sixteen (16) antigenic SAPs each, and eleven (11) were detected by IgA as antigenic. The IgG and IgM of patient AB1002 recognized a higher number of antigens (16) than IgM and IgA of patient's sera AB1001 and AB1003.

Nine (9) *A. baumannii* antigenic SAPs were recognized by all the three Igs isotypes (77.8, 66.2, 48.7, 40.9, 34.4, 26.0, 25.0, 23.0 and 18.0 kDa). Among them are 40.9 and 34.4 kDa, which were reported as exclusive antigenic SAPs to *A. baumannii*(in another study, data not shown here). The important features of the 40.9 and 34.4 kDa proteins are that they had minimal cross-reactivity with non-*Acinetobacter* sera. The 48.7 kDa was also detected by all the three Igs with minimal cross-reactivity to non-*Acinetobacter* sera, although not exclusive SAP to *A. baumannii* in SDS-PAGE. Three (3) antigenic SAPs (70.3, 21.0 and 14.0 kDa) were recognized exclusively by IgM. The IgG and IgM recognised four (4) antigenic SAPs (62.0, 46.0, 32.0, and 16.0 kDa). The IgG and IgA recognised two antigenic SAPs (96.7 and 85.2 kDa). While IgG exclusively recognised one antigenic SAP (30.0 kDa).

Cross-reactivity with non-*Acinetobacter* sera, *A. baumannii* antigenic SAPs showed minimal cross-reactivity with non-*Acinetobacter* sera six (6) antigenic SAPs (66.2, 62.0, 48.7, 40.9, 34.4, and 16.0 kDa) detected by IgG, in six (6) antigenic SAPs (48.7, 46.0, 34.4, 21.0, 16.0, and 14.0 kDa) detected by IgM and in ten (10) antigenic SAPs (96.7, 85.2, 77.8, 48.7, 40.9, 34.4, 26.0, 25.0, 23.0 and 18.0 kDa) detected by IgA. All the three Igs isotypes detected the 48.7, 40.9 and 34.4 kDa with minimal cross-reactivity to non-*Acinetobacter* sera (except that the 40.9 kDa SAP cross-reacted with IgM of many non-*Acinetobacter* sera).

	Molecular weight of SAPs bands	Antigenic SAPs detected by IgG			Antigenic SAPs detected by IgM			Antigenic SAPs detected by IgA			Number of cross-reacting non- <i>Acinetobacter</i> sera			Non- <i>Acinetobacter</i> sei
		AB1001	AB1002	AB1003	AB1001	AB1002	AB1003	AB1001	AB1002	AB1003	IgG	IgM	IgA	
1	96.7 kDa	+++	+++	++NB.....			0	+	0	8	NB	0	a. <i>Pseudomonas aerogenosa</i> b. <i>Klebsiella pneumoniae</i> c. <i>Salmonella</i> species d. <i>Escherichia coli</i> e. <i>Staphylococcus aureus</i> o. <i>Candida albicans</i> g. <i>Bacillus</i> species h. <i>Enterobacter</i> species
2	85.2 kDa	+++	+++	+++NB.....			0	+	0	8	NB	0	
3	77.8 kDa	++	++	+	+	++	+	+	+	0	8	8	3	
4	70.3 kDaNB.....			+	++	+NB.....			NB	8	NB	
5	66.2 kDa	++	+	+	++	++	++	0	0	++	1	8	4	
6	62.0 kDa	++	++	+	++	+++	+NB.....			1	8	NB	
7	48.7 kDa	+	+	+	+	+	+	+++	+++	+++	3	3	0	
8	46.0 kDa	+	++	+	+	+	+NB.....			8	1	NB	
9	40.9 kDa	+	++	+	+++	+++	+++	0	0	++	1	8	1	
10	34.4 kDa	+	++	+	+	+	+	0	+	0	1	1	0	
11	32.0 kDa	+	++	+	++	++	+NB.....			8	6	NB	
12	30.0 kDa	+++	+++	+++NB.....		NB.....			8	NB	NB	
13	26.0 kDa	+	+	++	+++	+++	+++	0	0	+	8	6	2	
14	25.0 kDa	+++	+++	+++	+++	+++	+++	0	+	++	8	6	0	
15	23.0 kDa	+++	+++	++	+++	+++	++	0	0	++	8	6	2	
16	21.0 kDaNB.....			++	+++	0NB.....			NB	3	NB	
17	18.0 kDa	0	+	0	+++	+++	+++	0	0	+	6	5	0	
18	16.0 kDa	0	++	0	0	+	0NB.....			1	2	NB	
19	14.0 kDaNB.....			++	++	0NB.....			NB	1	NB	
TOTAL		16			16			11						

Key: NB: No antigenic band detected, Numbers: indicate number of cross-reacting non-*Acinetobacter* sera with antigenic SAPs,

+ / ++ / +++: Indicate the intensity of SAP antigenicity.

- Antigenic SAPs detected by IgG, IgM and IgA
- Antigenic SAPs detected exclusively by IgG
- Antigenic SAPs detected by IgM only
- Antigenic SAPs detected by IgG and IgM

LC-MS/MS Identification of antigenic SAPs of *A. baumannii*

The objective of this part of the work was to characterize the selected surface antigenic SAPs biomarkers from *A. baumannii* expressed at 37°C using Liquid Chromatography-Mass Spectrometry (LC-MS/MS). The antigenic SAPs that were exclusive to *A. baumannii* (34.4 and 40.9 kDa) were first resolved via 10% SDS-PAGE and visualized with Coomassie Blue staining (Fig 2). The bands were then precisely excised from the gel, sliced into pieces, and sent for LC-MS/MS characterization. During LC-MS/MS, protein samples were briefly digested, and peptides extracted according to standard techniques (Bringans *et al.* Proteomics 2008). Peptides were analysed by electrospray ionisation mass spectrometry using the Shimadzu Prominence nano HPLC system [Shimadzu] coupled to a 5600 TripleTOF mass spectrometer [Sciex]. Tryptic peptides were loaded onto an Agilent Zorbax 300SB-C18, 3.5 µm (Agilent Technologies) and separated with a linear gradient of water/acetonitrile/0.1% formic acid (v/v). Spectra were analyzed to identify proteins of interest using Mascot sequence matching software (Matrix Science) with the SwissProt database.

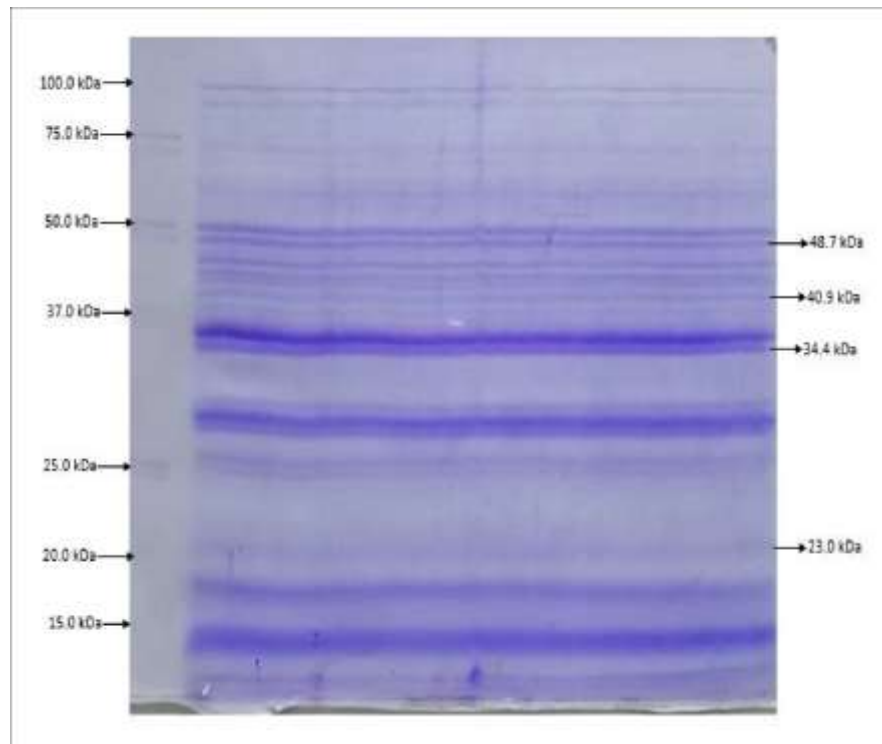


Figure 2: Preparative SDS-PAGE profile of SAPs of *A. baumannii* clinical isolate
*Arrow is indicating the targeted antigenic proteins for LC-MS/MS analysis.

Identification of 34.4 kDa from *A. baumannii* using mass spectrometry

The identity of *A. baumannii* 34.4 kDa proteins has been reported in previous studies as *A. baumannii* OmpA. One of these studies is conducted on *A. baumannii* OMPs in search of rapid biomarkers for the rapid detection of *A. baumannii* by Hasnat *et al.* (2011). The profiles of the OMPs of *A. baumannii* using SDS-PAGE and Western blot revealed specific antigen protein bands, 34.4 kDa, and further analysis suggests that the 34.4 kDa band corresponding to OmpA protein and was an excellent immunogen that could serve as a diagnostic biomarker for *A. baumannii* detection (Hasnat *et al.* 2011). Another study on *A. baumannii* OMPs using MALDI-TOF MS reported OMP 34.4 kDa as immunogenic proteins involved in virulence, antibiotic resistance, and bacterial growth (Chiang *et al.*, 2015a, and Chiang, *et al* 2020b).

LC-MS/MS analysis for 40.9 kDa from *A. baumannii*

The Mascot search results of 40.9 kDa revealed a score of 362, which means identity or extensive similarity at a significant level ($p < 0.05$) (<http://www.matrixscience.com>). A total of twelve (12) proteins matching the Tryptic digested peptides were recognised (Table 2). The molecular weights of the Tryptic digested peptides were compared against the protein database (Mascot, Matrix Science), and eight (8) peptides were identified in the amino acid sequence (Figure 2). The amino acids in bold were the peptides detected in the LC-MS/MS analysis that led to the identification of the 40.9 kDa protein as *A. baumannii* outer membrane protein Omp38. The *A. baumannii* Omp38 has been described as the potential virulence factor for inducing apoptosis of epithelial cells in the early stage of *A. baumannii* infection (Choi *et al.*, 2005, Al-Khalidi, *et al.*; 2026).

Table 2: Mascot Search Results of the proteins match of the tryptic digested peptides for *Acinetobacter baumannii* 40.9 kDa

Query	Molecular weight	Score	Peptides
224	869.4283	42	R. VFFDTNK
286	900.5029	67	R. LNDALSLR.T
387	950.5185	60	R. VFATITGSR.T
733	1106.5185	60	R. RVFATITGSR.T
765	1120.5513	53	K. LSEYPNATAR.I
1156	1311.6823	22	R. VFFDTNKSNIK.D
1243	1357.7313	38	R. LNDALSLRTEAR.A
1405	1436.6895	95	K. SALVNEYNVDSR.L
1571	1532.8198	64	K. SNIKDQYKPEIAK.V
1603	1547.7943	60	K. VAEKLSEYPNATAR.I
1767	1711.8781	58	K. QINGNFYVTSDLITK.N
1805	1789.8635	82	R. LSTQGFQWDQPIADNK.T
Total	12		

1	MKLSRIALAT NNGGKDGHLT	MLVAAPLAAA	NAGVTVTPLL	LGYTFQDSQH
51	NGPELQDDLF AGAAYKQKQ IQI	VGAALGIELT	PWLGFEAEYN	QVKGDVVDGAS
101	NGNFYVTSDL TRGTSEEGTL	ITKNYDSKIK	PYVLLGAGHY	KYDFDGVNRG
151	GNAGVGAFWR NVVLGGHLKP	LNDALSLRTE	ARATYNADEE	FWNYTALAGL
201	AAPVVEVAPV IKDQYKPEIA	EPTPVTPQPQ	ELTEDLNMEL	RVFFDTNKS
251	KVAEKLSEYP KSALVNEYNV	NATARIEGHT	DNTGPRKLNE	RLSLARANSV
301	DASRLSTQGF VVVQPGQEA	AWDQPIADNK	TKEGRAMNRR	VFATITGSRT
351	APAAAQ			

Figure 2 Matched peptides in bold, the amino acid sequences detected by LCMS/MS that led to identifying 40.9 kDa as *A. baumannii* Omp38.

DISCUSSION

Hospital-associated infections (HAIs) are secondary infections that develop within 48 hours of hospital admission, three days of hospital discharge, or 30 days following a surgical operation (Haque *et al.*, 2018). HAIs are responsible for increasing the duration of patient hospital stay and healthcare expenses. Patients with HAIs spent two and half times longer in hospital, with additional costs than uninfected patients (Gallagher & Baker, 2020). HAIs have been reported as an important cause of hospital morbidity and mortality around the world.

Acinetobacter baumannii ranked the top priority healthcare problem bacteria causing HAIs worldwide, particularly in the hospital's ICUs (López-Durán, *et al.*; 2020). *Acinetobacter baumannii* is responsible for more than 40% of nosocomial pneumonia and blood stream infections, mostly in immunocompromised patients (Karah *et al.*, 2020). The high prevalence of *Acinetobacter baumannii* HAIs coupled with the delay in time taken to produce Laboratory results has direct relation with high mortality rate (Doughari *et al.*, 2011; Almasaudi *et al.*; 2016). This necessitates the need for search new quick and easy diagnostic methods for *Acinetobacter* nosocomial infections in clinical laboratories.

This study is the first to report the complete comparative analysis of the SDS-PAGE SAPs profiling of *A. baumannii*. The SDS-PAGE analysis of the SAPs of *A. baumannii* ATCC 19606, *A. baumannii*, stains revealed a total of 22 SAP bands with molecular weight ranged from 14.0 to 104.0 kDa.

Analysis of SAPs profiles expressed by *A. baumannii*, showed that the two isolates expressed 46.4% of SAP bands. The SDS-PAGE SAPs profiling revealed that the low molecular weight SAPs in *A. baumannii* appeared to be highly expressed.

A study on the *A. baumannii* heat-shock proteins and the effects of varying temperatures on the *A. baumannii* SAP's expression revealed that *A. baumannii* 34.4, and 40.9 kDa expression might be related to the *A. baumannii* survival strategies to harsh environmental conditions as they were highly expressed at high temperature in an *in vitro* study and were not expressed at low temperature in the same study. Similarly, *A. baumannii* 34.4 kDa was reported to play a significant role in evading host immune response in animal rat model study of *A. baumannii* pathogenesis (Méndez, *et al.*, 2015; Severin *et al.*, 2020. Janet-Maitre *et al.*; 2025).

The SAPs profile analysis between *A. baumannii* ATCC 19606 and *A. baumannii* clinical isolates showed that additional two SAPs (70.3 and 18.0 kDa) were observed in the *A. baumannii* clinical strain that was not seen in reference strain of *A. baumannii* ATCC 19606 reference strain SAP profiles. The occurrence of these two more SAPs may be related to the patient's immune status since the reference strain did not

express these additional two SAPs. They were only seen in the strain isolated from a patient. Studies have shown that some surface proteins were only expressed in response to the extracellular environment changes (Cincarova *et al.*, 2016). The 70.3 and 18.0 kDa were also reported in the previous study while investigating the effects of temperature variation on *A. baumannii* SAP's expression. This study's findings have further unveiled the presence of SAP bands (40.9 and 34.4 kDa), which are unique to *A. baumannii*, as it is the first study to demonstrate these unique SAPs possessed by *A. baumannii*. The expression of unique SAPs may be used as a basis for the phenotypic identification and differentiation of the members of the *A. baumannii* complex.

The Western blot was performed to analyze the host humoral immune response in *Acinetobacter* infection patients against the specific antigens in the SAPs.

Western blot analysis showed variation in host immune response in all the sera tested. These individual variations in antibody response showed the differences in the host humoral immune system's ability to respond against the antigen present in the SAPs. In *A. baumannii* ATCC19606 reference strain, the antigenicity study showed that all the SAPs seen in SDS-PAGE were antigenic, while in *A. baumannii* clinical isolate, 86.4% of the SAPs from SDS-PAGE profiles were antigenic. IgG and IgM detected a higher percentage of 84.2% each, followed by IgA 57.9%.

Among them, IgG detected a higher number (88.8%), IgA (77.8%), and IgM (61.1%).

Antibody level against a specific pathogen's epitope rises gradually following the infection by the pathogen. The level of IgG in serum begins to increase in the second weeks at the onset of bacterial infection and slowly reaches its peak, and remain detectable for months or, in some s, life case (Magnus *et al.*, 2022). IgG is often detected in patients' sera with bloodstream infections, even after recovery (Oberhelman *et al.*, 1991; RasolofoRazanamparany *et al.*, 2001; Phaliponet *et al.*, 2002; Jennison&Verma, 2004).

Detection of a high percentage of antigenic SAPs in this study by the IgG and IgM suggest that these antigens can be promising biomarker candidates for the rapid detection of *A. baumannii*. The IgM antibodies are the first immunoglobulins produced during a humoral immune response. The IgM levels increased during the first week of illness. Then, by two weeks, the IgM levels reached maximum plasma level and began to decline to basal levels in most patients (Gupta *et al.*, 2023). Immunoglobulin M (IgM) accounts for about 5 to 10% of human immunoglobulins in the blood (1.5mg/ml) (Racine & Winslow, 2009). However, IgM has a higher affinity for antigens than IgG, related to the IgM'spentameric structure. (Jacofsky *et al.*, 2020; Panda & Ding, 2015).

Immunoglobulin A (IgA) is the first line of defense against invading pathogens in the mucosal lining tracks (Corthesy *et al.*, 2013). The total IgA in serum increased within 2 to 4 days at the early stage of the disease and peaked within the first week of the disease progression (Rasolofo-Razanamparany *et al.*, 2001).

In *A. baumannii* high molecular weight antigenic SAPs showed high antigenicity compared to their lower molecular weight SAPs. Increased expression of these low molecular weight SAPs in SDS-PAGE and their corresponding detection as highly antigenic in Western blot may suggest that they may play certain roles in the bacterial host immune response.

This study observed cross-reactivity between non-*Acinetobacter* sera and *A. baumannii*, *A.* antigenic SAPs. However, this is not surprising as it is a common phenomenon among closely related bacterial genera (Pantophl *et et al.*, 2001). Overall, most *A. baumannii*, antigenic SAPs showed minimal cross-reactivity with the IgA of non-*Acinetobacter* sera than does IgG and IgM.

Furthermore, in this study, the *A. baumannii* exclusive antigenic, 40.9 and 34.4 kDa were recognized by the IgG, IgM, and IgA of patient sera positive for *A. baumannii* infection. These findings indicated that these unique antigenic SAPs maybe be in the rapid identification and differentiation of *A. baumannii*.

Identification of *A. baumannii*, *A. nosocomialis*, and *A. pittii* selected biomarker antigenic SAPs by Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

This part of the study aimed to determine the identity of the selected biomarker antigens of *A. baumannii*, *A. nosocomialis*, and *A. pittii* using LC-MS/MS. The LCMS/MS technique has been used as a convenient and reliable method for bacterial proteomic studies, including *Acinetobacter* (Wang *et al.*, 2017). The *A.*

baumannii 34.4 kDa identification by LC-MS/MS was reported in the previous studies. The findings revealed its identity as OmpA (Kurupatiet *et al.*, 2006; Nafariehet *et al.*, 2017; Oh *et al.*, 2020), while in this study, the *A. baumannii* 40.9 kDa was identified as Omp38, the *A. baumannii* 23.0 kDa as a ribosome-recycling factor (RRF), and the *A. baumannii* 48.7 kDa, as the elongation factor protein.

The *A. baumannii* OmpA has been proven experimentally to play a significant role in the apoptosis of epithelial cells in the early stage of *A. baumannii* infection. The OmpA outer membrane is critically required for the formation and stabilization of biofilm on the epithelial cells to induce apoptosis (Brossard & Campagnari, 2012). Moreover, the *A. baumannii* OmpA vaccine with an adjuvant (aluminum hydroxide) has been shown to produce antibodies against OmpA with a high titer, as determined through animal models of systemic *A. baumannii* infectious (Huang *et al.* 2016). Sun *et al.* (2022) reported increased IgG levels against OmpA in the immuno-protective studies in mice. These are strong indications that *A. baumannii* OmpA could be used as a candidate diagnostic biomarker for *A. baumannii* detection. As far as the available literature this study was able to reach, this is the first report on the identity of *A. baumannii* 48.7, 40.9, and 23.0 kDa as elongation factor protein, Omp38, and ribosome-recycling factor, respectively.

The translation elongation factor Tuf is a moonlighting plasminogen-binding protein exposed on the outer surface of *A. baumannii*. The binding to Tuf depends Plasminogen, once bound to Tuf, can be converted to active plasmin and proteolytically degrade fibrinogen and the key complement component C3b. Thus, Tuf acts as a multifunctional protein that may contribute to the virulence of *A. baumannii* by aiding in the dissemination and evasion of the complement system. In comparison, the Omp38 has been reported to have the potential to induce apoptosis of epithelial cells in the early stage of *A. baumannii* infection. Ribosomes Recycling Factor is involved in dissociating 70S ribosomes into subunits in vivo. Inactivation of RRF in vivo leads to the accumulation of 70S ribosomes. These 70S ribosomes can still translate poly (U) (Hirokawa *et al.*, 2004; Moll *et al.*, 2004).

On the other hand, the LC-MS/MS identification of *A. nosocomialis* 55.1 kDa in this study revealed its identity as a chaperonin protein. Chaperonin was reported in some gram-negative bacteria in the previous studies. It played an essential role in environmental adaptation and virulence in Gram-negative bacteria by modulating stress responses, surface architectures, and virulence factors (Kuo *et al.*, 2017). Similarly, the *A. nosocomialis* 33.0 kDa protein was identified by LC-MS/MS in this study as a 30S ribosomal protein. This protein is one of the protein subunits of the ribosome, machinery of bacterial protein synthesis. The *A. nosocomialis* 48.7 kDa protein was identified as ATP synthetase subunit beta protein. Again, this is the first study to report the identity of these exclusive antigenic SAPs (55.1, 33.0) of *A. nosocomialis*.

The LC-MS/MS identification of *A. pittii* selected antigenic SAPs showed the 43.0 kDa protein elongation factors. The elongation factor Tu protein is a G protein that catalyzes the binding of aminoacyl-tRNA to the A-site of the ribosome inside living cells (Koenigs *et al.*, 2015). Likewise, the LC-MS/MS analysis 35.0 kDa protein identified it as *Acinetobacter* outer membrane protein Omp38. The *Acinetobacter* Omp38 has been described as the potential virulence factor for inducing apoptosis of epithelial cells in the early stage of infection (Choi *et al.*, 2005). The LC-MS/MS analysis identified the 48.7 kDa protein as outer membrane protein *omp38* or ATP synthase subunit alpha. Also, the LC-MS/MS identified the *A. pittii* 23.0 kDa protein as a Ribosome-recycling factor (RRF). The RRF is a protein involved in disassembling the 70S ribosomes (the working form of ribosomes) into subunits for the initiation of the next round of protein synthesis (Hirokawa *et al.*, 2005). More importantly, the presence of 48.7 and 23.0 kDa antigenic SAPs in all the three species (*A. baumannii*, *A. nosocomialis*, and *A. pittii*) revealed that these common antigens might be used as a candidate biomarker for the rapid diagnosis of any member of the ACB complex.

CONCLUSIONS

The present study has successfully profiled complete *A. baumannii*, *A. nosocomialis*, and *A. pittii* surface membrane proteins, and determined their antigenicity by Western blot analysis. From the available literature, this study is the first of its kind to profile the SAPs of all three clinically important

Acinetobacter species. A large number of the previous studies were dedicated to *A. baumannii* alone with more focused-on the *A. baumannii* OMPs. This study also successfully identified presence of SAPs that were exclusive present in *A. baumannii* (40.9, 36.0, and 34.4 kDa), *A. nosocomialis* (55.1 and 33.0 kDa) and *A. pittii* (43.0 and 35.0 kDa). Two SAPs were identified to be common to all the three *Acinetobacter* species (48.7 and 23.0 kDa). The study also revealed that the three bacterial species of the ACB complex group shared 46.4% of the SAPs that are common between the three species studied. The SDS-PAGE SAP profiling also revealed that the exclusive SAP bands detected in the *A. baumannii* and *A. pittii* were highly expressed, suggesting their high antigenicity as excellent diagnostic biomarkers.

The study also successfully characterized those exclusive SAPs and those SAPs common to all the three species by LC-MS/MS mass spectrometry. The identification and characterization of the antigenic SAPs exclusive to each member of the ACB complex and antigenic SAPs common to all members of the ACB complex provided a platform for the development of a rapid diagnostic test for the early diagnosis of *Acinetobacter* infection and differentiation of *A. baumannii*, *A. nosocomialis* and *A. pittii* infection. Early diagnosis of *Acinetobacter* HAIs involved the detection of *Acinetobacter* antigens or antibodies against *Acinetobacter* in the patient's serum as early as possible so that the patient has the best chance for successful treatment. When *Acinetobacter* HAIs diagnosis is delayed, there is a lower chance of patient survival, greater problems associated with treatment, and higher health care costs. Early diagnosis improves patient outcomes by providing care at the earliest possible stage and is an important public health strategy in all hospital settings.

The finding from this work showed that the *Acinetobacter* SAPs are excellent potential diagnostic and vaccines biomarker candidates. This was evidenced by the detection of a high percentage of antigenic SAPs by the IgG and IgM of patient sera positive for *Acinetobacter* infections, including the findings of exclusive antigenic SAPs (40.9, 36.0, and 34.4 kDa unique to *A. baumannii*, 55.1 and 33.0 kDa unique to *A. nosocomialis*, and 43.0 and 35.0 kDa unique to *A. pittii*, and antigenic SAPs 48.7 and 23.0 kDa common to all the three species).

RECOMMENDATIONS

Further studies on the species-exclusive SAPs need to be carried out using ELISA to evaluate the diagnostic performance of these proteins. A larger sample size of patient's sera infected with *A. baumannii*, *A. nosocomialis*, and *A. pittii* need to be used to further confirm the diagnostic performance of the selected proteins. The combined effect for the identified target proteins needs to be studied to enhance their potential use as protein-based diagnostic markers. In addition, the protective immune response of these identified proteins can also be investigated to evaluate their usefulness as vaccine candidates.

Although the Western blot assay was more sensitive in detecting antigens or antibodies, its use as a routine diagnostic assay has several downsides. These included the requirement for trained personnel, technically laborious, inconsistency in electrotransfer of antigens from SDS-PAGE to the membrane, and unsuitability to screen a larger number of sera. Therefore, the biomarkers identified in this study can be further developed in the form of ICT (immunochromatography) test, EIA (enzyme immunoassay) test, or PCR (polymerase chain reaction) test, which can be very helpful in diagnosing patients with *Acinetobacter* infection. Furthermore, up-scaling of these biomarker SAPs may be achieved via amino-acid sequencing and cloning of the respective genes encoding the target proteins. Nevertheless, peptide-based immunoassay may have added advantage to reduce the cross-reaction compared to the use of the full length of the antigenic protein.

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