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Diagnostic evaluation of LTA and suPAR as combined biomarkers for multidrug-resistant Gram-positive infections: a case-control study in Iraq

Mohammed Sadeq Khalife[1,*], May Mohammed Ali[1], Sawsan M. Jabbar Al-Hasnawi[1], Basit Khaled Hawas[2]

[1]Department of Medical Microbiology, College of Medicine, University of Kerbala, Karbala, Iraq
[2]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Kerbala, Karbala, Iraq

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*** CORRESPONDING AUTHOR:**
Mohammed Sadeq Khalife, Department of Medical Microbiology, College of Medicine, University of Kerbala, Karbala, Iraq; e-mail: mohammed.sadeq@s.uokerbala.edu.iq

ABSTRACT

Antimicrobial resistance (AMR) has emerged as a critical threat to global public health. Timely and accurate identification of resistant pathogens (followed by appropriate antimicrobial therapy and robust stewardship) is essential in order to curb the emergence and spread of AMR. Although current diagnostic modalities offer clinical value, they are constrained by time requirements and limited capacity to differentiate colonization from active infection. Lipoteichoic acid (LTA), specific to Gram-positive bacteria, serves as a direct indicator of bacterial presence. In parallel, the soluble urokinase-type plasminogen activator receptor (suPAR) reflects host immune response, providing insight into infection severity and progression. This study investigates the diagnostic and prognostic utility of a dual biomarker panel comprising LTA and suPAR for the rapid assessment of bacterial infections. In a case–control design, we have quantified the serum levels of LTA and suPAR by ELISA in 62 patients with confirmed bacterial infections and 38 matched controls. Statistically significant elevations were observed in patients with extensively drug-resistant (XDR) infections: LTA levels reached 54.47 ng/L (p=0.0111), and suPAR levels reached 284.74 ng/mL (p=0.0019), compared to the multidrug resistant (MDR) infection and the non-MDR infection groups. These findings underscore the potential of LTA and suPAR as complementary biomarkers for the early detection and stratification of bacterial infections.

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1. Introduction

Major antimicrobial resistance (AMR) represents a critical global health challenge, driven largely by the overuse and misuse of antibiotics. Inappropriate antibiotic application across agricultural, medical, and veterinary practice fosters the emergence of resistance genes, contributing to a "silent pandemic" projected to surpass other leading causes of mortality by 2050[1]. The proliferation of antibiotic-resistant microorganisms poses a substantial threat to global morbidity and mortality. Given its profound public health and socioeconomic implications, AMR has long been recognized by the World Health Organization (WHO) as a priority area for pharmaceutical innovation. In 2016, WHO member states requested the development of a priority list of antibiotic-resistant bacteria to guide research and drug development efforts[2].

In the intensive care unit (ICU), *Staphylococcus aureus* is frequently implicated in sepsis, ventilator-associated pneumonia, surgical site infections, and infections related to indwelling medical devices. The widespread emergence of methicillin-resistant *S. aureus* (MRSA) has led to the classification of many ICU staphylococcal infections as drug-resistant. Additionally, coagulase-negative staphylococci (CoNS), including *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and more recently *S. lugdunensis*, have become prominent pathogens in healthcare-associated infections[3]. Across hospitals and urban centers, the treatment of bacterial infections caused by antibiotic-resistant strains presents an escalating clinical challenge[4].

Antimicrobial susceptibility testing remains essential for selecting appropriate antimicrobial agents in the management of infectious diseases. Diagnostic microbiology laboratories have seen continuous methodological evolution. Traditional phenotypic approaches such as disc diffusion and broth microdilution, though labour-intensive and time-consuming, are still regarded as gold standards. In response to clinical demands, research efforts increasingly focus on developing rapid susceptibility testing methods suitable for routine laboratory use. These innovations often incorporate automation and leverage genotypic or micro- / nanotechnological platforms[5].

Terminology such as multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) is commonly employed in order to describe the spectrum of resistance patterns observed in healthcare-associated bacterial infections. Notably, infections caused by multidrug-resistant organisms often present with clinical features indistinguishable from those caused by susceptible strains[6].

Lipoteichoic acid (LTA), a surface-associated amphiphilic molecule involved in regulating autolytic wall enzymes (muramidases), is produced by Gram-positive bacteria. Upon bacteriolysis (induced by β-lactam antibiotics, lysozyme, or leukocyte-derived cationic peptides), LTA is released and may persist within macrophages, contributing to chronic inflammation[7]. The soluble urokinase-type plasminogen activator receptor (suPAR), the bioactive form of the membrane-bound glycoprotein uPAR, is predominantly expressed on immunologically active cells. As a marker of local inflammation and immune activation, suPAR has gained recognition as a predictive biomarker in various inflammatory conditions[8].

This study aimed at evaluating a biomarker panel comprising LTA and suPAR for the detection of Gram-positive MDR bacterial infections, with the goal of advancing rapid and accurate diagnostic strategies for these clinically challenging pathogens.

2. Methodology

A case–control study was conducted between October and December 2024 at the Al-Husseini Teaching Hospital and the Al-Kafeel Specialized Hospital, Iraq. The study included 62 patients with confirmed bacterial infections and 38 healthy controls. Diagnoses were based on clinical signs and symptoms observed by physicians at the Al-Imam Al-Hussain Hospital, under the Karbala Health Directorate.

Among the 62 cases of Gram-positive bacterial infections, the distribution was as follows: sepsis (N=27), diabetic foot ulcers (N=8), urinary tract infections (N=9), wound infections (N=8), vaginitis (N=3), abscesses (N=3), and pneumonia (N=4). In-

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Table 1. Comparison of biomarker levels of lipoteichoic acid (LTA) and soluble urokinase-type plasminogen activator receptor (suPAR), measured in ng/L and ng/mL, respectively, across control and patient groups or stratified by antimicrobial resistance patterns. Abbreviations used: MDR, multidrug resistant; SD, standard deviation; UTI, urinary tract infection; XDR, extensively drug resistant.

Variable		LTA		suPAR	
		Mean	SD	Mean	SD
Bacterial infection types	Control	15.27	7.17	120.67	23.68
	Sepsis	47.68	9.98	231.97	84.18
	UTI	47.32	8.44	250.49	82.32
	Diabetic foot ulcer	45.86	4.66	243.53	55.15
	Pneumonia	44.59	12.98	197.77	50.67
	Vaginitis	43.12	4.39	300.47	113.35
	Wound infection	60.62	9.44	257.05	107.77
	Abscess	29.94	4.54	191.8	39.46
***p*-value**		0.0002		0.0001	
Antimicrobial resistance patterns	Non-MDR	43.86	11.32	199.38	47.25
	MDR	48.91	9.09	260.44	87.60
	XDR	54.47	8.57	284.74	96.63
***p*-value**		0.0111		0.0019	

clusion criteria were based on physician-confirmed bacterial infections in patients aged 18 years or older, of either gender. Exclusion criteria encompassed individuals under 18 years of age, those with autoimmune diseases, pregnant women, patients with catheters, and males with prostate conditions.

Specimen collection included urine, blood, and swab samples for microbial isolation and identification. A loopful of each sample was inoculated onto MacConkey and blood agar plates, followed by overnight incubation at 37°C under aerobic conditions. Blood samples were also collected so as to quantify LTA and suPAR levels in both patient and control groups.

Microbial identification and antimicrobial susceptibility testing were performed using the VITEK 2 system (bioMérieux), employing the ID-GPC identification card and AST modules. Ethical approval was obtained from the Ethics Committees of the Kerbela Health Office, with institutional consent from the Imam Al-Hussain Hospital and the Al-Kafeel Specialized Hospital.

Statistical analysis was conducted by using the SPSS version 23 software. Mean and standard deviation values were calculated, and statistical significance was assessed using a threshold of $p<0.05$. Analysis of variance (ANOVA) was applied for group comparisons, with Duncan's *post hoc* test being used in order to evaluate multiple comparisons at the same significance level.

3. Results and Discussion

All infection groups demonstrated significantly elevated LTA levels compared to controls (15.27 ± 7.17 ng/L; $p=0.0002$). Among the infection types, wound infections exhibited the highest mean LTA concentration (60.62 ± 9.44 ng/L), followed by sepsis (47.68 ± 9.98 ng/L), urinary tract infections (47.32 ± 8.44 ng/L), diabetic foot ulcers (45.86 ± 4.66 ng/L), pneumonia (44.59 ± 12.98 ng/L), and vaginitis (43.12 ± 4.39 ng/L), with abscesses showing the lowest, yet still elevated levels (29.94 ± 4.54 ng/L) (Table 1).

Similarly, suPAR levels were markedly elevat-

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ed across all infection groups compared to controls (120.67 ± 23.68 ng/mL; p=0.0001). Vaginitis showed the highest mean suPAR concentration, followed by wound infections (257.05 ± 107.77 ng/mL), urinary tract infections (250.49 ± 82.32 ng/mL), diabetic foot ulcers (243.53 ± 55.15 ng/mL), and sepsis (231.97 ± 84.18 ng/mL), while pneumonia (197.77 ± 50.67 ng/mL) and abscesses (191.85 ± 39.46 ng/mL) exhibited the lowest, yet still elevated levels (Table 1).

In terms of resistance patterns, LTA levels were significantly higher in XDR cases (54.47 ng/L; p=0.0111) compared to non-MDR (43.86 ng/L) and MDR (48.91 ng/L) groups. suPAR levels were also significantly (p=0.0019) elevated in XDR (284.74 ng/mL) and MDR (260.44 ng/mL) cases relative to non-MDR infections (199.38 ng/mL) (Table 1).

These findings corroborate previous studies. For instance, elevated LTA levels have been demonstrated in bacterial infections, even in surgically treated mice without bacterial inoculation[8]. Elevated suPAR levels across all infection groups align with the findings of a previous study[9], which has reported that high plasma suPAR concentrations may predict case fatality and severe sepsis in emergency department patients with suspected infection. Furthermore, our results support the diagnostic and prognostic utility of LTA and suPAR in bacterial infections and antimicrobial resistance profiling[10].

4. Conclusion

In conclusion, the significant elevation of LTA and suPAR in patients with bacterial infections underscores their potential as diagnostic and prognostic biomarkers. These findings contribute to the growing body of evidence supporting the role of these molecules in the host immune response to bacterial pathogens. Future research should aim at elucidating the underlying mechanisms of these biomarker elevations and explore their therapeutic implications in clinical practice.

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Conflicts of interest

None exist.

ORCIDs

0009-0003-9015-5427 (M.S. Khalife); 0000-0001-6999-497X (M.M. Ali); 0000-0002-5715-0697 (S.M.J. Al-Hasnawi); 0009-0007-4258-4832 (B.K. Hawas)

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