

Direct identification of bacteria from positive blood culture bottles by VITEK-MS: evaluation of two lysis-centrifugation methods

Abdul Rehman, Irim Iftikhar, Hijab Batool, Karam Rasool

Chughtai Institute of Pathology, Lahore Pakistan

ABSTRACT

Background: Bloodstream infections cause significant global morbidity and mortality, and although MALDI-TOF MS accelerates pathogen identification, it still requires subculture. This study evaluated two in-house lysis-centrifugation methods (LCMs) using sodium dodecyl sulfate (SDS) and Triton X-100 for direct pathogen identification from positive blood culture bottles. To compare the diagnostic accuracy of SDS- and Triton X-100-based LCMs for direct bacterial identification from positive blood culture bottles using VITEK MS.

Material and Method: This study was conducted at the Chughtai Institute of Pathology, Lahore. Positive blood culture bottles were analyzed using the standard culture-based method and both LCM protocols in parallel. Blood cultures with polymicrobial growth, yeast on Gram stain, or no identification by the standard-of-care method were excluded. Concordance, diagnostic accuracy, and processing time were assessed.

Results: A total of 251 monobacterial positive blood cultures were analyzed. The SDS and Triton X-100 protocols correctly identified 62.5% and 59.8% of isolates, respectively ($p = 0.52$). Concordance was higher for Enterobacterales (66.7%) than for non-Enterobacterales (50.0% and 32.1%, respectively). Identification rates for *Salmonella enterica* were significantly lower than for other Enterobacterales ($p < 0.001$). Both methods required less than 15 minutes of hands-on time per sample and showed fair agreement ($\kappa \approx 0.31$).

Conclusion: Both LCMs provided rapid, low-cost identification with comparable performance. Their utility was greater for Enterobacterales other than *S. enterica*, while low *S. enterica* identification rates limit their application for rapid enteric fever diagnosis.

Key words: Blood culture, MALDI-TOF, Sepsis, Bloodstream infection, Lysis centrifugation.

BACKGROUND

Blood stream infections (BSI) including sepsis are a major cause of morbidity and mortality all over the world and their incidence has shown an increasing trend over the years.¹ World health organization (WHO) reported 49 million global cases of sepsis and 11 million related potentially avoidable deaths in 2017.² Enteric fever and infectious endocarditis are other significant blood stream infections for which routine blood cultures are performed. Enteric fever caused by *Salmonella enterica* serotypes Typhi and Paratyphi is an especially important issue in Pakistan due to high rates of extensively drug resistant (XDR) *S. Typhi* strains.³

Correspondence: Dr. Abdul Rehman, Resident Microbiology, Chughtai Institute of Pathology, Lahore Pakistan

Email: abdulrehman66458@gmail.com

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Prompt and accurate identification in blood cultures can help physicians to initiate appropriate management timely.⁴ Blood culture remains the gold standard for establishing the diagnosis and guiding towards most appropriate management in blood stream infections. However, it is limited by low sensitivity and poor turnaround time.⁵

Recent advancements in blood culture incubation techniques and rapid identification methodologies have made marked improvement in the turnaround time of blood cultures. Multiple culture independent methodologies utilizing multiplex PCRs are available for rapid identification of pathogens and susceptibility however their routine utility is limited by scarcity of available clinical data.⁶ Clinical use of Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry MALDI-TOF MS for pathogen identification has made significant impact in patient management and prognosis with a 23% reduction in patient mortality, 5.07-h reduction in time to effective antibiotic therapy and 22.86-h reduction in time to identify microorganisms.⁷ However routine MALDI-TOF analysis requires culturing of positive blood culture bottles on solid media to obtain visible

colonies which usually take 18-24 hours for most pathogens and up to 72 hours for slow growing bacteria and yeasts. To reduce this delay in identification multiple in-house and commercial kits have been developed to extract pathogens from positive blood cultures using Lysis-Centrifugation, Selective Filtration and serum separator techniques and utilize them for direct identification.⁸

Available commercial kits include the Sepsityper® Kit (Bruker Daltonics, Bremen, Germany), VITEK® MS Blood Culture Kit (bioMérieux) and rapid BACpro® II (Nittobo Medical Co., Tokyo, Japan). Although the commercial kits demonstrate good results and applicability, they are expensive and arduous to perform with multiple extraction steps. This has prompted researchers to explore and develop simpler and more cost-effective in-house protocols to extract pathogens. These in-house protocols have utilized selective lysis of red cells by ionic or nonionic surfactants such as Sodium dodecyl sulphate (SDS)⁹, Triton-X100^{10,11}, and Ammonium Chloride¹² to varying outcomes. Other modalities used include saponin¹³ and serum separator tubes.¹⁴

Recent studies from South Asia have also evaluated direct MALDI-TOF MS identification from positive blood culture broth. A recent study from Pakistan employed a simple in-house separator gel tube and saline wash protocol, demonstrating good performance for Gram-negative bacteria while emphasizing its low cost and ease of implementation in routine laboratories.¹⁵ Similarly, studies from India have evaluated both detergent-based in-house lysis protocols (using SDS, Triton X-100, and saponin) and commercial kit-based approaches, including the VITEK® MS Blood Culture Kit.^{16,17} These studies reported reduced turnaround times and good diagnostic performance, particularly for Gram-negative organisms, while highlighting that in-house methods are considerably less expensive and require only routinely available laboratory reagents, whereas commercial kits offer greater standardization at a substantially higher cost. Although extensive research has been done on this subject in the western world, Australia and China, there is still limited published data from South Asia, particularly evaluating the performance of detergent-based in-house lysis-centrifugation protocols in diverse patient populations, including patients with enteric fever. This study aimed to check the utility of these

methods in both in-patient and out-patient settings including their applicability in the rapid diagnosis of *Salmonella* species causing enteric fever. For this study we opted for LCM using SDS and Triton X-100 due to simple procedures and promising results as demonstrated by recent studies^{9, 11} and evaluated their diagnostic accuracy, time effectiveness and practical utility.

MATERIAL AND METHODS

The study was conducted from 1st July 2023 to 30th December 2023 in the Microbiology department of Chughtai Institute of Pathology, Lahore. A commercial laboratory which receives clinical samples of both indoor and outdoor patients from multiple collection centers across Pakistan. All aerobic (BacT/ALERT® FA Plus), anaerobic (BacT/ALERT® FN Plus) and pediatric (BacT/ALERT® PF Plus) blood culture bottles were incubated in the automated incubator (BacT/ALERT® VIRTUO™) at 37° C for 5 days. Positive blood culture bottles were examined by Gram stain (Scharlau, Barcelona, Spain). Only bottles showing a single bacterial morphotype (monobacterial growth) were eligible for inclusion. Bottles showing yeast on Gram stain, polymicrobial growth, duplicate positive blood cultures from the same patient within one week or isolates that could not be identified by the standard-of-care (SOC) method were excluded. Yeasts were excluded because the lysis-centrifugation protocols evaluated in this study were developed and validated for bacterial identification, and MALDI-TOF MS direct identification of yeasts from blood culture broth generally requires modified extraction procedures.

To include sufficient number of gram-negative isolates including *S. enterica*, other Enterobacterales and non-Enterobacterales, we opted for consecutive purposive sampling targeting a gram-negative to gram-positive ratio of 3 to 1. During the study period, 270 unique positive bottles were initially selected, 19 bottles were then subsequently excluded due to polymicrobial growth or no identification by SOC method (Figure I). All selected samples were analyzed by standard of care (SOC) culture-based identification method and the direct lysis-centrifugation methods in parallel.

Positive blood culture bottles were inoculated on chocolate agar (Oxoid, Hampshire, United Kingdom), blood agar (Oxoid, Hampshire, United Kingdom), and MacConkey agar (Oxoid, Hampshire, United Kingdom)

plates and incubated aerobically and anaerobically (Anaerobic bottles) for 16-24 hours or till sufficient growth was obtained. A single colony was applied to a single well of a VITEK® MS-DS target slide (bioMérieux). One microliter of matrix (VITEK® MS-CHCA, [3.1g/100µl alpha-Cyano-4-hydroxycinnamic acid]) (bioMérieux) was overlaid and the slide was air dried. The slide was analyzed in the VITEK® MS [Software Version 1.0.0.46] (bioMérieux) and results with confidence level > 99% were used for

identification. Additionally serotyping (Pro_Lab diagnostics, United Kingdom) was done for isolates identified as *Salmonella enterica subspecies enterica*. The LCMs were performed in batches of 6 blood culture bottles following the protocols devised by their respective authors^{9,11} and are outlined in Figure 2A-2B. The pellets obtained were then applied for MALDI-TOF analyses (Figure 2C). Identifications with a confidence of >95% in any of the spots were considered accurate.

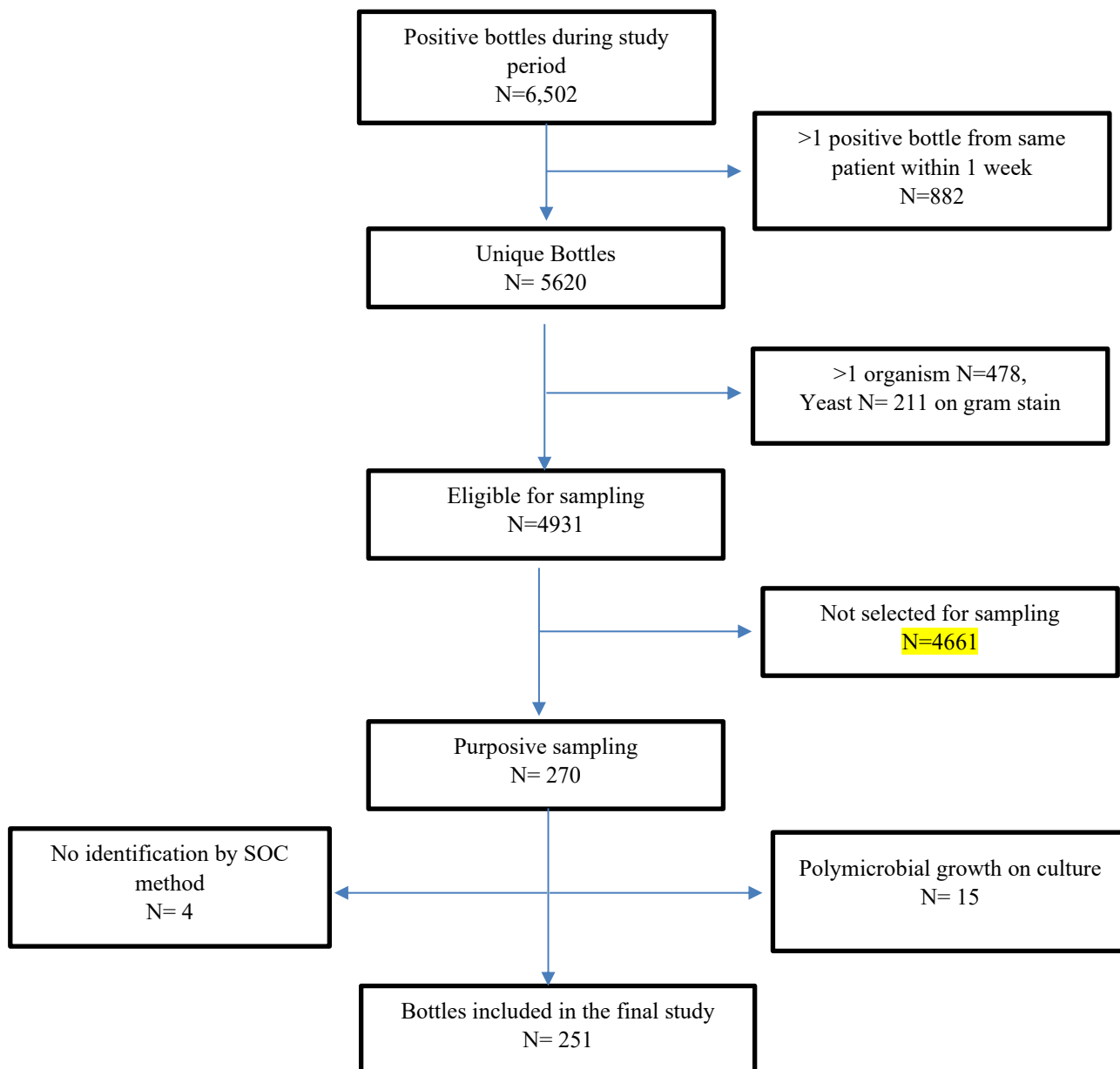


Figure-I: Flowchart outlining the selection of positive blood culture bottles for study.

SOC: Standard of care

Direct Identification Workflow from Positive Blood Culture by VITEK-MS

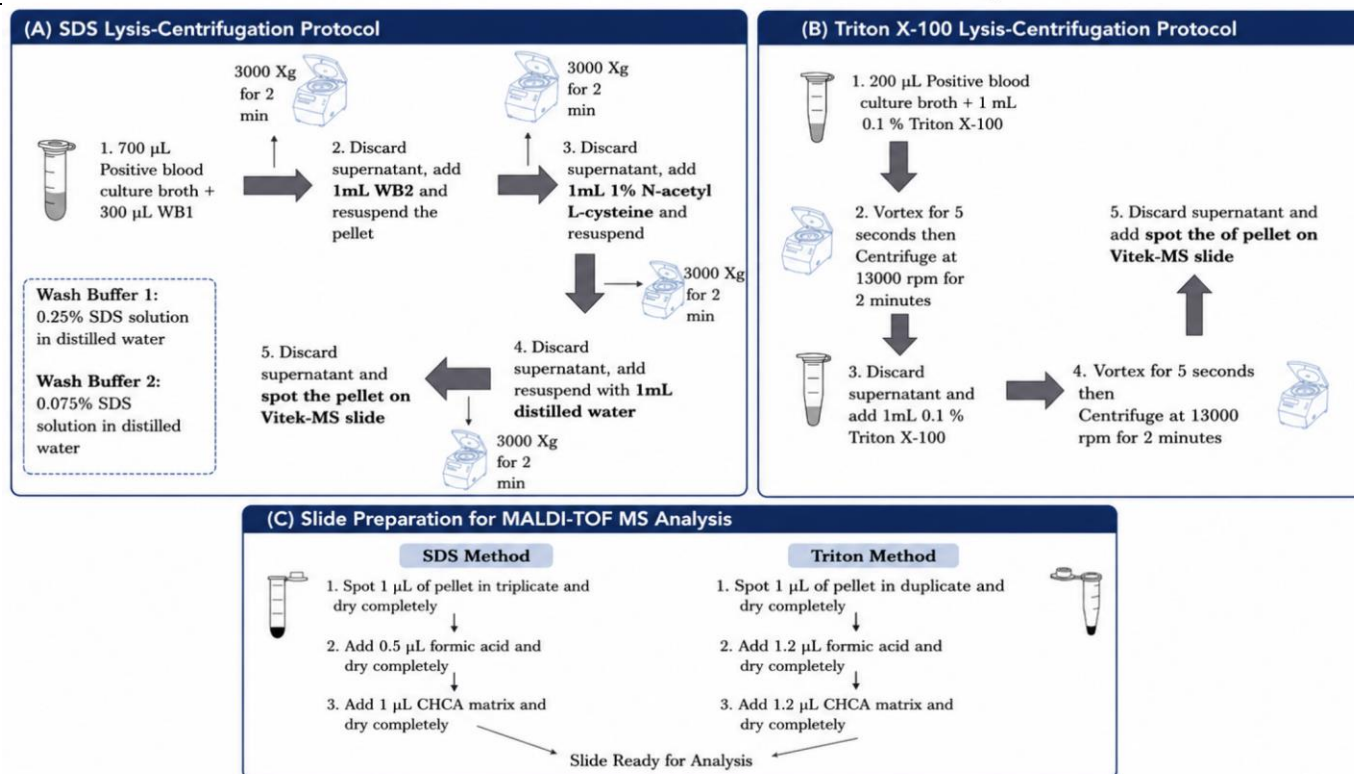


Figure 2. Workflow of direct MALDI-TOF MS identification from positive blood culture. (A) SDS lysis-centrifugation protocol based on Forster et al. [9]. (B) Triton X-100 lysis-centrifugation protocol based on Simon et al. [11]. (C) Slide preparation for MALDI-TOF MS analysis. WB1, Wash Buffer 1; WB2, Wash Buffer 2; CHCA, α -cyano-4-hydroxycinnamic acid.

RESULTS

During the study period, 6,502 blood culture bottles flagged positive (Figure-I). After excluding duplicate positive bottles from the same patient within one week ($n = 882$), 5,620 unique positive bottles remained. Of these, 478 bottles showing polymicrobial organisms on Gram stain and 211 bottles showing yeast were excluded, leaving 4,931 bottles eligible for sampling. A total of 270 bottles were selected by purposive sampling. Subsequently, 15 bottles were excluded because polymicrobial growth was detected on subculture and four because no organism was identified by the standard-of-care method, resulting in a final study population of 251 monobacterial bacterial isolates. Positive blood culture bottles included 128 (51.0%) pediatric, 80 (31.9%) aerobic, and 43 (17.1%) anaerobic blood culture bottles. Standard-of-care (SOC) identification yielded 36 bacterial species belonging to 23 genera, including 184 (73.3%) Gram-negative and 67 (26.7%) Gram-positive isolates. The most frequently isolated organisms were *Salmonella enterica* ($n = 78$), *Klebsiella pneumoniae* ($n = 43$), *Escherichia coli* ($n = 24$), *Staphylococcus hominis* ($n = 23$), and *Staphylococcus aureus* ($n = 17$) (Table I & II). The SDS lysis-centrifugation method correctly identified 157/251 (62.5%) isolates to species level,

whereas the Triton X-100 method correctly identified 150/251 (59.8%) isolates. The difference in overall concordance between the two methods was not statistically significant ($p = 0.52$). Sample processing required approximately 15 minutes per sample (30 minutes per batch of six) for the SDS protocol and 10 minutes per sample (20 minutes per batch of six) for the Triton X-100 protocol.

SDS LCM correctly identified 157 out of 251 isolates to the species level (Table-I). Among the gram-negative rods, the concordance rate for Enterobacterales was 66.67% (104/156) compared to 50% for non-Enterobacterales (14/28). Among the Enterobacterales, concordant results were obtained for 36/78 (46.1%) *S. enterica* isolates as compared to 68/78 (87.1%) for the rest of the Enterobacterales. For gram-positive bacteria, concordant results were obtained for 35/47 (74.4%) Staphylococci, compared to 3/15 (20%) for Streptococci and 1/5 (20%) for gram-positive rods. 1 (0.4%) isolate was correctly identified to genus level (*Burkholderia contaminans* as *B. cepacia*), 2 (0.8%) isolates were misidentified (1 *Staphylococcus aureus* as *Bacillus psychrosaccharolyticus* and 1 *Salmonella enterica* as *Enterobacter cloacae*). Thus, correct identifications were made for 98.03 % of samples which achieved

identifications with confidence of >95%, and no identification was made for 91(36.3%) bottles.

Triton LCM accurately identified 150 out of 251 isolates at the species level (Table-II). For gram-negative rods, the concordance rate for Enterobacterales was 66.67% (104/156), while for non-Enterobacterales it was 32.14% (9/28). Among the Enterobacterales, concordant results were obtained for 39/78 (50%) *S. enterica* isolates, compared to 65/78 (83.33%) for other Enterobacterales. For gram-positive bacteria, concordant results were observed in 30/47 (63.8%) *Staphylococci*, 6/15 (40%) for *Streptococci*, and 1/5 (20%) for gram-positive rods. One (0.4%) isolate was identified to the genus level (*Streptococcus dysgalactiae* as *S. equii*), while two (0.8%) isolates were misidentified (*Ochrobactrum intermedium* as *Mycobacterium genavense* and *Bacillus cereus* as *Neisseria mucosa*). Thus, correct identifications

were made for 98.03 % of samples which achieved identifications with confidence of >95%, additionally, no identification was made in 98 (39%) of the blood culture bottles.

The overall concordance did not differ significantly between the two methods (62.5% vs 59.8%, $p = 0.52$), nor did it differ within any subgroup, including Gram-negative bacteria, Enterobacterales, non-Enterobacterales, Gram-positive bacteria, *Staphylococci*, *Streptococci* and Gram-positive rods (Table-III). For both methods, concordance was significantly lower for *Salmonella enterica* than for the other Enterobacterales: 36/78 (46.1%) vs 68/78 (87.2%) with SDS, and 39/78 (50.0%) vs 65/78 (83.3%) with Triton X-100 ($p < 0.001$ for both; Table-IV).

Table-I: Results obtained by SDS LCM protocol.

Routine I.D	Identified by SOC method (n)	Correct identification (n)	Correct upto Genus (n)	Misidentified (n)	No ID (n)	Concordance (%)
Gram-Negative Bacteria	184	118	1	1	64	64.1%
Enterobacterales	156	104	0	1	51	66.67%
<i>Salmonella enterica</i>	78	36	0	1	41	46.1%
<i>Escherichia coli</i>	24	18	0	0	6	75%
<i>Klebsiella pneumoniae</i>	43	41	0	0	2	95.3%
<i>Serratia marcescens</i>	5	5	0	0	0	100%
<i>Enterobacter cloacae</i> complex	4	3	0	0	1	75%
<i>Providencia rettgeri</i>	1	0	0	0	1	0
<i>Cronobacter sakazakii</i>	1	1	0	0	0	100%
Non-Enterobacterales	28	14	1	0	13	50%
<i>Achromobacter denitrificans</i>	1	1	0	0	0	100%
<i>Acinetobacter baumannii</i>	6	4	0	0	2	66.67%
<i>Acinetobacter pittii</i>	1	1	0	0	0	100%
<i>Aeromonas punctata/caviae</i>	1	1	0	0	0	100%
<i>Burkholderia cepacia</i>	6	3	0	0	3	50%
<i>Burkholderia cenocapacia</i>	4	1	0	0	3	25%
<i>Burkholderia contaminans</i>	1	0	1	0	0	-
<i>Ochrobactrum intermedium</i>	1	0	0	0	1	-
<i>Pseudomonas aeruginosa</i>	4	2	0	0	2	50%
<i>Pseudomonas stutzeri</i>	1	0	0	0	1	-
<i>Stenotrophomonas maltophilia</i>	2	1	0	0	1	50%
Gram-Positive Bacteria	67	39	0	1	27	58.20%
Staphylococci & related genera	47	35	0	1	11	74.4%
<i>Staphylococcus aureus</i>	17	12	0	1	4	70.5%
<i>Staphylococcus epidermidis</i>	1	1	0	0	0	100%
<i>Staphylococcus haemolyticus</i>	4	2	0	0	2	50%
<i>Staphylococcus hominis</i>	23	18	0	0	5	78.26%

<i>Staphylococcus gallinarum</i>	1	1	0	0	0	100%
<i>Micrococcus luteus</i>	1	1	0	0	0	100%
Streptococci & related genera	15	3	0	0	12	20%
<i>Aerococcus viridans</i>	2	0	0	0	2	-
<i>Enterococcus faecium</i>	1	0	0	0	1	-
<i>Enterococcus faecalis</i>	3	1	0	0	2	33.3%
<i>Granulicatella adiacens</i>	1	0	0	0	1	-
<i>Streptococcus dysgalactiae</i>	1	0	0	0	1	-
<i>Streptococcus gordonii</i>	1	0	0	0	1	-
<i>Streptococcus mitis/oralis</i>	4	1	0	0	3	25%
<i>Streptococcus pneumoniae</i>	1	0	0	0	1	-
<i>Streptococcus pyogenes</i>	1	1	0	0	0	100%
Gram-positive rods	5	1	0	0	4	20%
<i>Bacillus cereus</i>	1	0	0	0	1	-
<i>Microbacterium arborescens</i>	1	1	0	0	0	100%
<i>Listeria monocytogenes</i>	3	0	0	0	3	0
Total	251	157	1	2	91	62.54%

Table-II. Results obtained by Triton X-100 LCM protocol.

Routine I.D	Identified on SOC method (n)	Correct identification (n)	Correct upto Genus (n)	Misidentified (n)	No ID (n)	Concordance (%)
Gram-Negative Bacteria	184	113	0	1	70	61.4%
Enterobacterales	156	104	0	0	52	66.67%
<i>Salmonella enterica</i>	78	39	0	0	39	50%
<i>Escherichia coli</i>	24	22	0	0	2	91.6%
<i>Klebsiella pneumoniae</i>	43	35	0	0	8	81.3%
<i>Serratia marcescens</i>	5	3	0	0	2	60%
<i>Enterobacter cloacae complex</i>	4	3	0	0	1	75%
<i>Providencia rettgeri</i>	1	1	0	0	0	100%
<i>Cronobacter sakazakii</i>	1	1	0	0	0	100%
Non Enterobacterales	28	9	0	1	18	32.1%
<i>Achromobacter denitrificans</i>	1	0	0	0	1	-
<i>Acinetobacter baumannii</i>	6	3	0	0	3	50%
<i>Acinetobacter pittii</i>	1	1	0	0	0	100%
<i>Aeromonas punctata/caviae</i>	1	1	0	0	0	100%
<i>Burkholderia cepacia</i>	6	0	0	0	6	-
<i>Burkholderia cenopacia</i>	4	0	0	0	4	-
<i>Burkholderia contaminans</i>	1	0	0	0	1	-
<i>Ochrobactrum intermedium</i>	1	0	0	1	0	-
<i>Pseudomonas aeruginosa</i>	4	3	0	0	1	75%
<i>Pseudomonas stutzeri</i>	1	0	0	0	1	-
<i>Stenotrophomonas maltophilia</i>	2	1	0	0	1	50%
Gram-Positive Bacteria	67	37	1	1	28	55.2%
Staphylococci & related genera	47	30	0	0	17	63.8%
<i>Staphylococcus aureus</i>	17	9	0	0	8	52.9%
<i>Staphylococcus epidermidis</i>	1	1	0	0	0	100%
<i>Staphylococcus haemolyticus</i>	4	3	0	0	1	75%
<i>Staphylococcus hominis</i>	23	15	0	0	8	65.2%
<i>Staphylococcus gallinarum</i>	1	1	0	0	0	100%
<i>Micrococcus luteus</i>	1	1	0	0	0	100%
Streptococci & related genera	15	6	1	0	8	40%
<i>Aerococcus viridans</i>	2	1	0	0	1	50%
<i>Enterococcus faecium</i>	1	1	0	0	0	100%
<i>Enterococcus faecalis</i>	3	1	0	0	2	33.3%
<i>Granulicatella adiacens</i>	1	1	0	0	0	100%
<i>Streptococcus dysgalactiae</i>	1	0	1	0	0	-
<i>Streptococcus gordonii</i>	1	0	0	0	1	-
<i>Streptococcus mitis/oralis</i>	4	1	0	0	3	25%
<i>Streptococcus pneumoniae</i>	1	0	0	0	1	-

<i>Streptococcus pyogenes</i>	1	1	0	0	0	100%
Gram-positive rods	5	1	0	1	3	20%
<i>Bacillus cereus</i>	1	0	0	1	0	-
<i>Microbacterium arborescens</i>	1	0	0	0	1	-
<i>Listeria monocytogenes</i>	3	1	0	0	2	33.3%
Total	251	150	1	2	98	59.8%

Table-III: Comparison of SDS and triton X-100 LCM results.

	Number of isolates	Concordant results by SDS LCM	Concordant results by Triton-X100 LCM	p-value
Total	251	157(62.54%)	150(59.8%)	.5215
Gram-Negative Bacteria	184	118(64.1%)	113(61.4%)	.5897
Enterobacterales	156	104(66.67%)	104(66.67%)	1
Non-Enterobacterales	28	14(50%)	9(32.14%)	.1744
Gram-Positive Bacteria	67	39(58.2%)	37(55.22%)	.1216
Staphylococci	47	35(74.4%)	30(63.8%)	.2641
Streptococci	15	3(20%)	6(40%)	.2318
Gram-Positive rods	5	1(20%)	1(20%)	1

Table-IV: Comparison of results for *Salmonella enterica* and other Enterobacterales.

LCM Method	Concordant results		p-value
	<i>Salmonella enterica</i>	Other Enterobacterales	
SDS	36/78	68/78	<0.00001
Triton-X100	39/78	65/78	0.00001

DISCUSSION

Multiple studies have been done in the last decade exploring different in-house methodologies to isolate pathogens from positive blood culture bottles that can then be utilized directly for identification by MALDI-TOF. Most researchers opted for selective lysis of blood cells followed by centrifugation and washing steps to obtain a bacterial pellet that can be spotted onto MALDI-TOF slides directly. There is considerable variation amongst the results, but overall Gram-negative bacteria have been identified with a much higher success as compared to Gram-positive bacteria and fungi.^{9, 11, 12, 14, 18}

Both the lysis centrifugation methods provided rapid results with comparable accuracies (Table-III) and the identification rates to species level were like the trends in previous studies with higher identification rates for gram-negative bacteria than gram-positive bacteria, and very low identification rates for *Streptococci*. There was fair agreement between the SDS and Triton-X100 LCMs ($\kappa \approx 0.31$). However, the correct identification rates for both methodologies were significantly lower than those achieved in the original research. The SDS protocol by Forster *et al.*, 2022 correctly identified 198/266 (74.4%) pathogens to species level identifying 87/96 (91.4%) gram-negative bacteria and 110/167 (65.9%) gram-positive bacteria.⁹ The Triton X-100 protocol developed by Loic Simon *et*

al., correctly identified 80.5% (632 total isolates) of bacteria up to species level with a higher percentage of Gram-negative bacteria correctly identified (90.5%) as compared to Gram-positive bacteria (80.5%).¹¹

There can be multiple plausible causes for the lower identification rates. Previous studies were limited to a particular hospital department, a single hospital or a selected hospital as compared to our study which included samples from multiple inpatient and outpatient settings with variable sample collection protocols and diverse patient populations. Furthermore, our most common isolate *Salmonella enterica* had identification rates that were considerably lower compared to other members of Enterobacterales by both methodologies (Table-IV).

This finding has important clinical implications in Pakistan, where enteric fever remains endemic and *Salmonella enterica* serovar Typhi is a common cause of bloodstream infection, including multidrug-resistant and extensively drug-resistant (XDR) strains. Delayed or failed identification may postpone organism-directed antimicrobial therapy and public health interventions. Recently, a study from Pakistan evaluated a simplified in-house direct MALDI-TOF protocol using separator-gel tube centrifugation and saline washing, demonstrating good performance for Gram-negative bacteria but lower identification rates for Gram-positive organisms. However, unlike our study, it did not include

a large number of *S. enterica* isolates and therefore did not assess the performance of direct MALDI-TOF for enteric fever.¹⁵

Although rapid identification via VITEK-MS significantly shortens the time-to-genus/species diagnosis, it does not provide phenotypic susceptibility. Consequently, conventional gold-standard methods like broth microdilution remain imperative for guiding definitive therapy in XDR cases.

CONCLUSION

Our study demonstrates that the rapid Lysis centrifugation protocols can be useful tools for identifying agents causing bloodstream infection in the right settings and the identification can then be used for rapid susceptibility testing. Both the protocols produced comparable results with similar hands-on time. The identification rates for Enterobacterales other than *Salmonella enterica* were very high therefore they can have significant clinical application for rapid diagnosis of gram-negative septicemia. However, the low identification rates for *S. enterica* limit their applicability in outpatient settings in our population for the rapid diagnosis of enteric fever.

Additional research is warranted to confirm our findings and fine tuning of the protocols for improvement of identification rates in Gram-positive bacteria and *Salmonella enterica*.

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DISCLOSURES

None

CONFLICT OF INTEREST

None

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Declared none

AUTHOR CONTRIBUTION

Abdul Rehman: Data curation, investigation, formal analysis, original draft, final approval, accountable for all aspects of publication

Irim Iftikhar: Conceptualization, methodology, supervision, project administration, review, final approval, accountable for all aspects of publication

Hijab Batool: Methodology, review and editing, Formal analysis, final approval, accountable for all aspects of publication

Karam Rasool: Resources, validation and project administration, final approval, accountable for all aspects of publication

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