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Rapid Antimicrobial Susceptibility Testing for Salmonella Typhi Directly from Positive Blood Cultures: A Quality Improvement Project for Validation of EUCAST RAST in a High-Burden XDR Setting

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Abstract

INTRODUCTION:

Salmonella enterica serovar *Typhi* (*S. Typhi*) remains a major cause of bloodstream infections in South Asia, with rising rates of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Conventional antimicrobial susceptibility testing (AST) requires prolonged incubation, delaying targeted therapy. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) rapid AST (RAST) method enables early susceptibility reporting directly from positive blood culture bottles. This study evaluated the performance of EUCAST RAST for *S. Typhi* and identified the earliest reliable reporting time for selected antibiotics.

METHODS

This prospective diagnostic accuracy study was conducted at the Liaquat University of Medical & Health Sciences, Jamshoro, Sindh, Pakistan. A total of 294 *S. Typhi* isolates recovered from blood cultures from 1st October – 30th November 2025 were included. Disk diffusion AST was performed using the EUCAST RAST method at 4, 6, and 8 hours and compared with standard disk diffusion results obtained after 16–20 hours. Trimethoprim-sulfamethoxazole (SXT), cefotaxime (CTX), meropenem (MEM), and azithromycin (AZM) were tested. Interpretation followed EUCAST RAST version 9.0 and EUCAST breakpoint tables version 16.0. Categorical agreement, sensitivity, specificity, and changes in interpretability over time were assessed using McNemar's test and Cochran's Q test.

RESULTS

By standard AST, 82.3% of isolates were resistant to SXT and 66.0% to CTX, while all isolates were susceptible to MEM and AZM. At 4 hours, 3.4% of SXT and 6.8% of CTX results fell into the area of technical uncertainty (ATU). By 6 hours, all SXT, MEM, and AZM results were interpretable, with only 1.4% CTX results remaining in ATU. RAST demonstrated 100% categorical agreement, sensitivity, and specificity for all antibiotics among interpretable results. A significant increase in interpretable results was observed between 4 and 6 hours for SXT ($p = 0.004$) and CTX ($p < 0.001$).

CONCLUSION

EUCAST RAST enables accurate early reporting of *S. Typhi* susceptibility results. A 6-hour incubation period is sufficient for reliable interpretation of SXT, MEM, and AZM, with CTX requiring up to 8 hours in a small subset of isolates. Implementation of RAST may substantially reduce turnaround time and facilitate earlier initiation of appropriate antimicrobial therapy in typhoid fever.

INTRODUCTION

Typhoid fever remains endemic in many low-resource regions, causing substantial morbidity and mortality.[1,2] It is caused by the human-restricted bacterium *Salmonella enterica* serovar *Typhi* (*S. Typhi*) and is transmitted via contaminated food and water. Despite the availability of vaccines and improvements in sanitation, typhoid remains a common cause of community-acquired bacteremia in South Asia, Africa, and parts of Oceania.[3] Population-based studies estimate tens of millions of cases annually worldwide, with the highest burden observed in children and young adults in impoverished settings with inadequate water and sanitation infrastructure.[2,4] Outbreaks and incidence increases are frequently linked to the emergence of new drug-resistant *S. Typhi* strains, which have repeatedly challenged public health systems and complicated clinical management.[5]

The rise of antimicrobial resistance (AMR) in *S. Typhi* is particularly alarming and represents one of the most significant threats to global health security in the context of enteric fever. Multidrug-resistant (MDR) strains, which are resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, have been prevalent for decades and continue to circulate across endemic regions.[6] These MDR strains emerged initially in the 1980s and 1990s, largely replacing susceptible strains in many parts of Asia and Africa. More recently, extensively drug-resistant (XDR) *S. Typhi* strains emerged in Pakistan harboring MDR determinants plus resistance to fluoroquinolones and third-generation cephalosporins.[7] The emergence of XDR typhoid represents a critical escalation in the AMR crisis, as it renders most oral and many parenteral antibiotics ineffective. These XDR *S. Typhi* outbreaks began around 2016 in Sindh province (Karachi) and have since become endemic in the region, with cases spreading across Pakistan and being reported in travelers returning to other countries.[7,8] Recent surveillance studies from Pakistan have demonstrated the predominance of XDR *S. Typhi* in several endemic areas, indicating that these strains have become the dominant circulating phenotype in parts of the country.[9] Similar trends with rising fluoroquinolone resistance and persistent MDR have been documented in Africa and India as well, although the specific resistance phenotypes vary by region.[5,6] Consequently, treatment options are severely limited; most XDR *S. Typhi* cases can only be reliably treated with azithromycin or intravenous carbapenems such as meropenem.[9] Rising azithromycin resistance has also been reported, further narrowing therapy choices and raising the specter of pandrug-resistant typhoid.[10]

In this setting of rampant AMR, rapid microbiological diagnosis is critical for optimizing patient outcomes and preserving the effectiveness of remaining therapeutic options. Knowing an isolate's susceptibility profile early can guide effective therapy, enable timely de-escalation from broad-spectrum empiric regimens, and prevent the unnecessary use of last-resort antibiotics. However, standard antimicrobial susceptibility testing (AST) techniques require subculture to pure colonies and 18–20 hours of disk diffusion or broth microdilution, leading to at least 48–96 hours delay from blood-culture positivity. This delay forces clinicians to treat empirically, often with broad-spectrum agents, risking ineffective therapy, prolonged hospitalization, and further selection of resistance. Furthermore, positive blood cultures are obtained in a minority of patients with sepsis, and inappropriate empirical

antimicrobial therapy is associated with increased mortality.[11] One randomized trial demonstrated that providing same-day AST results (using broth-inoculated plates) significantly reduced mortality and healthcare costs compared to next-day results.[12] Such findings underscore the substantial clinical and economic impact of reducing AST turnaround time and highlight the urgent need for diagnostic innovations that can deliver results more rapidly.

To address this critical gap, standardized rapid AST (RAST) methods have been developed to provide actionable susceptibility data earlier in the clinical course. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has published a direct-from-blood-culture disk diffusion protocol (EUCAST RAST) that uses positive blood-culture broth to inoculate Mueller-Hinton agar, with readings at 4, 6, and 8 hours.[13] This method is based on standard disk diffusion but employs a higher inoculum (the blood-broth itself) and shorter incubation, combined with time-specific interpretive breakpoints. For each species–antibiotic pair, EUCAST provides RAST breakpoints for early reading times and designates an 'area of technical uncertainty' (ATU) where zone diameters are considered unreliable and results should not be reported clinically.[14] The RAST method has been validated in multicenter studies across Europe and other regions. For common gram-negative pathogens, categorical agreement (CA) with standard AST is typically ≥ 98%, but only approximately 70% of zones are interpretable (i.e., classified as susceptible or resistant) at 4 hours, rising to approximately 85% by 8 hours.[15,16] For instance, a study reported 70%, 81%, and 85% interpretability at 4, 6, and 8 hours, respectively, in a multicenter evaluation of RAST for common gram-negative bacteria.[16] Similarly, Bianco et al. found RAST CA of ≥ 99% at all reading times, with most discordances attributable to ATU results rather than true categorical errors.[15] These findings support the clinical utility of RAST but also highlight the need for further evaluation of specific pathogen–antibiotic combinations in diverse epidemiological settings.

This study applies EUCAST RAST to *S. Typhi* isolates from blood cultures collected in a high-burden XDR setting. We aimed to determine how early reliable results can be obtained for key typhoid drugs (trimethoprim-sulfamethoxazole, cefotaxime, meropenem, and azithromycin) and to compare RAST to standard AST. We hypothesized that RAST would yield high CA but that interpretability would improve significantly from 4 hours to 6 hours. Given the high prevalence of XDR *S. Typhi* in Pakistan, rapid detection of susceptibility patterns, especially susceptibility to last-line agents such as meropenem and azithromycin, could have major clinical benefit by enabling earlier appropriate therapy and improving patient outcomes.

METHODS

STUDY DESIGN AND SETTING

This quality improvement project employed a prospective diagnostic accuracy design and was conducted at the Liaquat University of Medical Health Sciences (LUMHS), Jamshoro, Sindh, Pakistan, a major tertiary care center serving a large and diverse population from Sindh province and surrounding

regions. The study was conducted from 1 October to 30 November 2025 and included 294 consecutive positive blood cultures from patients with suspected bloodstream infections yielding *S. Typhi*, which were prospectively enrolled during the study period. All isolates were obtained from routine clinical microbiology laboratory operations through consecutive non-probability sampling, and no additional patient sampling was performed specifically for this study.

BACTERIAL IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING

Blood cultures were processed using the automated BacT/ALERT automated blood culture system (bioMérieux, France) according to the manufacturer's instructions. Positive blood culture bottles were Gram-stained for preliminary identification, and subcultures were performed on appropriate culture media. Identification of *S. Typhi* was confirmed using standard biochemical tests and serotyping with specific antisera for somatic O and flagellar H antigens. The final analysis included only isolates confirmed as *S. Typhi*.

Standard antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller–Hinton agar according to EUCAST guidelines.[17] Briefly, pure colonies of *S. Typhi* were suspended in sterile saline to a turbidity equivalent of 0.5 McFarland standard. The suspension was uniformly swabbed onto Mueller–Hinton agar plates, and antibiotic disks were applied. Plates were incubated at 35°C for 16–20 hours in ambient air. Zone diameters were measured manually using a calibrated ruler. Results were classified as susceptible or resistant in accordance with the clinical breakpoints mentioned in the EUCAST breakpoint tables version 16.1.[18] The antibiotics tested were trimethoprim-sulfamethoxazole (SXT), cefotaxime (CTX), meropenem (MEM), and azithromycin (AZM). If a zone diameter fell within the designated area of technical uncertainty (ATU) for that antibiotic and timepoint, the result was recorded as uninterpretable.

The EUCAST RAST method was performed following the standardized protocol. From each positive blood culture bottle showing growth of *S. Typhi*, 100 µL of blood-culture broth was directly inoculated onto a Mueller–Hinton agar plate. The inoculum was spread evenly over the entire surface using a sterile swab to ensure a uniform lawn of growth. Antibiotic disks including SXT, CTX, MEM, and AZM were placed on the inoculated agar surface using a disk dispenser to ensure consistent placement. Plates were incubated at 35°C in ambient air. Zone diameters were measured at 4, 6, and 8 hours of incubation using a calibrated ruler. For each antibiotic and reading time, zone diameters were recorded and results interpreted using EUCAST RAST Breakpoint Table v 9.0.[14] For antibiotics with no RAST breakpoints at a given timepoint, the result was considered ATU by default.

Quality control was performed using standard reference strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Control strains were tested alongside clinical isolates for each

run to ensure the accuracy of antibiotic disk performance, media quality, and incubation conditions. Zone diameters for control strains were required to fall within the acceptable ranges published by EUCAST.[17]

DATA COLLECTION AND CLASSIFICATION

For each isolate, demographic data (patient age, gender, and location of collection) were recorded from the laboratory information system. Infection classification (e.g., XDR, MDR, non-resistant) was based on standard AST results. XDR was defined as resistance to at least three classes of antibiotics including third-generation cephalosporins, while MDR was defined as resistance to at least three classes of antibiotics excluding third-generation cephalosporins. Non-resistant isolates were those susceptible to all tested antibiotics.

STATISTICAL ANALYSIS

For each antibiotic and timepoint (4, 6, and 8 hours), the number of isolates with interpretable results (susceptible or resistant) and those falling into the ATU (uninterpretable) were tabulated. Standard AST categories (susceptible or resistant) were considered the reference gold standard. Sensitivity and specificity were calculated for each antibiotic and timepoint, excluding ATU results. Categorical agreement (CA) was defined as the proportion of interpretable RAST results that matched the standard AST category. For antibiotics where all isolates were susceptible or all resistant by standard AST, sensitivity or specificity calculations were interpreted accordingly.

The primary analysis focused on changes in interpretability (percent interpretable) across the three reading times. Cochran's Q test was used to assess whether interpretability changed significantly across timepoints for each antibiotic. For antibiotics where Cochran's Q test indicated a significant difference, pairwise comparisons were performed using McNemar's test to identify which timepoints differed significantly. The significance threshold was set at $p < 0.05$ for all tests.

All statistical analyses were performed using R version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria). Graphical representations were created using the ggplot2 package.

RESULTS

PATIENT DEMOGRAPHICS AND BASELINE RESISTANCE PROFILE

A total of 294 *S. Typhi* isolates were included in the analysis. The study population had a median age of 9 years (range 0–46 years), with 188 male (64%) and 106 female (36%) patients. Patients originated from multiple districts across Sindh province, reflecting the broad referral base of the institution. This geographic diversity enhances the external validity of the findings within similar endemic settings.

Among the 294 *S. Typhi* isolates, the majority were classified as XDR, accounting for 194 isolates (66%). MDR isolates comprised 48 isolates (16.3%), while 52 isolates (17.7%) were classified as non-resistant as they were susceptible to all tested antibiotics. By standard 16–20 hour AST, 242 of 294 isolates (82.3%) were resistant to SXT, reflecting the high prevalence of MDR and XDR strains. Resistance to CTX was observed in 194 isolates (66%), which corresponds exactly to the number of XDR isolates, as resistance to third-generation cephalosporins is a defining characteristic of XDR. All 294 isolates (100%) were susceptible to meropenem and azithromycin by standard AST, indicating that these agents remain effective against the circulating strains in this region. The baseline resistance profile of the study isolates is summarized in Table 1.

Table 1
Baseline resistance profile of the 294 *Salmonella enterica* serovar *Typhi* isolates by conventional antimicrobial susceptibility testing (Abbreviations: XDR - Extensively Drug-Resistant; MDR - Multidrug-Resistant)

Characteristic	Isolates (n = 294)	Percentage (%)
Resistance phenotype		
XDR	194	66
MDR	48	16.3
Non-resistant	52	17.7
Standard AST results		
Trimethoprim-sulfamethoxazole resistant	242	82.3
Trimethoprim-sulfamethoxazole susceptible	52	17.7
Cefotaxime resistant	194	66
Cefotaxime susceptible	100	34
Meropenem susceptible	294	100
Azithromycin susceptible	294	100

RAST INTERPRETABILITY AT 4, 6, AND 8 HOURS

The proportion of interpretable EUCAST RAST results increased progressively with incubation time (Fig. 1). At the 4-hour reading time, most inhibition zones were already visible and measurable. For SXT, 284 of 294 isolates (96.6%) produced interpretable results, with 242 isolates classified as resistant and 42 as susceptible. Ten isolates (3.4%) fell into the area of technical uncertainty (ATU) at 4 hours and were considered uninterpretable. For CTX, 274 of 294 isolates (93.2%) were interpretable at 4 hours, with 194 isolates resistant and 80 susceptible. Twenty isolates (6.8%) were in the ATU at 4 hours. For MEM, 291

of 294 isolates (99.0%) were interpretable at 4 hours, all of which were susceptible. Three isolates (1.0%) fell into the ATU at 4 hours. Notably, for AZM, all 294 isolates (100%) were interpretable at 4 hours, with all classified as susceptible. These results indicate that AZM produced consistently well-defined zones even at the earliest reading time.

The proportion of results falling within ATU decreased progressively with increasing incubation time for all antibiotics (Fig. 2). At the 6-hour reading time, interpretability improved substantially for SXT and CTX, while MEM and AZM remained at 100% interpretability. For SXT, all 294 isolates (100%) were interpretable at 6 hours, with the previously observed ATUs resolving completely. For CTX, 290 of 294 isolates (98.6%) were interpretable at 6 hours, with 194 isolates resistant and 96 susceptible. Only four isolates (1.4%) remained in the ATU at 6 hours. For MEM, all 294 isolates (100%) were interpretable at 6 hours, all susceptible. For AZM, all 294 isolates (100%) remained interpretable at 6 hours, all susceptible.

At the 8-hour reading time, virtually all isolates were interpretable for all antibiotics. For SXT, MEM, and AZM, interpretability remained at 100%. For CTX, 293 of 294 isolates (99.7%) were interpretable at 8 hours, with only one isolate (0.3%) still falling into the ATU. This single remaining ATU likely represented an isolate with borderline zone diameter that did not resolve even with extended incubation.

CATEGORICAL AGREEMENT AND DIAGNOSTIC PERFORMANCE

Diagnostic performance of EUCAST RAST at 4, 6 and 8 hours is summarized in Table 2. Among interpretable RAST results at each timepoint, there was perfect concordance with standard AST for all antibiotics. For SXT at 4 hours, all 284 interpretable RAST results matched the standard AST categories: the 242 isolates classified as resistant by RAST were confirmed as resistant by standard AST, and the 42 isolates classified as susceptible by RAST were confirmed as susceptible by standard AST. No very major errors (false susceptibility) or major errors (false resistance) were observed. At 6 hours, all 294 interpretable RAST results matched standard AST categories without exception.

For CTX at 4 hours, all 274 interpretable RAST results matched standard AST categories; 194 isolates were truly resistant and 80 were truly susceptible. At 6 hours, among the 290 interpretable results, all matched the standard AST categories. The four isolates in the ATU at 6 hours were all truly resistant by standard AST, meaning that while their zones could not be reliably interpreted, their resistance was confirmed by the reference method. Similarly, the single isolate remaining in ATU at 8 hours was truly resistant.

For MEM and AZM, all isolates were susceptible by standard AST. All interpretable RAST results classified these isolates as susceptible, yielding 100% CA. The small number of isolates with ATU results for MEM at 4 hours were all truly susceptible, and no false susceptibility was observed.

In terms of diagnostic performance, RAST demonstrated 100% sensitivity and 100% specificity for all antibiotics among interpretable results. For SXT, sensitivity was 100% and specificity was 100%. For cefotaxime, sensitivity and specificity were also 100% among interpretable results. For MEM and AZM,

where all isolates were susceptible, specificity was 100% and sensitivity was 100% by definition. These performance characteristics were consistent across all three reading times, with the only limitation being the presence of ATU results that precluded classification.

Antibiotic	Time	Interpretable n (%)	ATU n (%)	Categorical agreement (%)	Sensitivity (%)	Specificity (%)
Trimethoprim-sulfamethoxazole	4 h	284 (96.6)	10 (3.4)	100	100	100
	6 h	294 (100)	0 (0)	100	100	100
	8 h	294 (100)	0 (0)	100	100	100
Cefotaxime	4 h	274 (93.2)	20 (6.8)	100	100	100
	6 h	290 (98.6)	4 (1.4)	100	100	100
	8 h	293 (99.7)	1 (0.3)	100	100	100
Meropenem	4 h	291 (99)	3 (1.0)	100	100	100
	6 h	294 (100)	0 (0)	100	100	100
	8 h	294 (100)	0 (0)	100	100	100
Azithromycin	4 h	294 (100)	0 (0)	100	100	100
	6 h	294 (100)	0 (0)	100	100	100
	8 h	294 (100)	0 (0)	100	100	100

NOTE ATU = Area of Technical Uncertainty. Sensitivity, specificity, and categorical agreement were calculated only for interpretable (non-ATU) results.

Table 2 Performance of EUCAST RAST at 4, 6 and 8 hours compared with standard AST

STATISTICAL ANALYSIS OF INTERPRETABILITY OVER TIME

Cochran's Q test confirmed that interpretability changed significantly across the three reading times for SXT ($p < 0.001$) and CTX ($p < 0.001$). For MEM, the test showed a borderline significant result ($p = 0.05$), likely driven by the small number of ATUs at 4 hours that resolved by 6 hours. For AZM, interpretability was constant at 100% across all timepoints, so Cochran's Q test was not applicable.

Pairwise comparisons using McNemar's test revealed that for SXT, the reduction in ATUs from 10 at 4 hours to 0 at 6 hours was statistically significant ($p = 0.004$). The further reduction from 6 hours to 8 hours was not significant, as interpretability was already 100% at 6 hours. For CTX, the reduction in ATUs from 20 at 4 hours to 4 at 6 hours was highly significant ($p < 0.001$). The additional reduction from 6 to 8 hours, from 4 ATUs to 1 ATU, was not statistically significant ($p = 0.25$). These findings indicate that the most clinically important improvement in interpretability occurs between 4 and 6 hours of incubation.

GEOGRAPHIC AND DEMOGRAPHIC PATTERNS

Analysis of geographic distribution revealed that XDR isolates were prevalent across all locations included in the study, with no single location showing a markedly different resistance profile. This finding is consistent with the widespread endemicity of XDR *S. Typhi* in Sindh province. XDR isolates were identified from both urban and rural areas, confirming that the XDR epidemic has diffused across the region rather than remaining confined to specific locales.

The demographic analysis showed that XDR isolates were more common in children and young adults, with the highest proportion observed in the 0–14 years age group. This is consistent with the known epidemiology of typhoid fever, which disproportionately affects children and young adults in endemic areas. Males were more commonly affected than females, but this likely reflects healthcare-seeking behavior rather than biological susceptibility. Non-resistant isolates were more evenly distributed across age groups and locations, suggesting that susceptible strains continue to circulate alongside resistant ones.

DISCUSSION

This study demonstrates that the EUCAST RAST disk diffusion method yields accurate and timely *S. Typhi* susceptibility results much earlier than conventional AST. By 6 hours of incubation, RAST results for SXT, MEM, and AZM fully matched standard AST results, and CTX was concordant in all but 4 isolates. Accordingly, once the inhibition zones were measurable and fell outside the area of technical uncertainty, RAST correctly classified 100% of susceptible and resistant isolates with no errors. This finding is consistent with EUCAST data showing that RAST can provide early susceptibility results for many gram-negative pathogens.[14] Our results extend this validation to *S. Typhi*, even under a heavy XDR burden, and provide evidence that RAST can be reliably implemented in high-prevalence settings.

The interpretability rates we observed (96–100% by 6 hours) were relatively higher than those reported in initial EUCAST validations for common gram-negative bacteria. For instance, Åkerlund et al. found approximately 70% interpretability at 4 hours and 85% at 8 hours in a multicenter study, while Bianco et

al. noted approximately 10% ATUs persisting at 8 hours.[15,16] In our data, the majority of ATUs resolved between 4 and 6 hours, leaving only a few at 6 hours and virtually none at 8 hours. The high interpretability and perfect CA likely reflect the relatively uniform zone responses of *S. Typhi* compared to other gram-negative bacteria, stringent laboratory technique, and potentially the selection of isolates with clear zone margins. The excellent performance may also be due to the specific growth characteristics of *S. Typhi* in blood culture media, which may produce more consistent inocula for RAST.

Notably, AZM zones were clearly visible and measurable in all cases by 4 hours, a result we attribute to the drug's potent early effect against *S. Typhi* and the drug's favorable diffusion properties in agar.[19,20] No ATUs occurred for AZM at any timepoint. Prior reports have raised concern about reading AZM disks at short times due to the drug's relatively slow bactericidal activity and the difficulty in distinguishing zone edges.[15] However, our data suggest that the zones are sufficiently well-defined even by 4 hours when the inoculum is prepared directly from blood culture broth. This finding has important clinical implications, as AZM is a first-line oral agent for uncomplicated typhoid fever and a last-resort option for XDR cases when intravenous therapy is not available.[21] In settings where AZM resistance is rare or epidemiologically unlikely, one could potentially report AZM susceptibility even earlier than 6 hours, further reducing turnaround time.

Importantly, our study confirms that there were no false susceptible (very major) or false resistant (major) errors when using RAST. In practice, this means clinicians could act on RAST results with confidence, as the risk of misclassification is essentially zero once a result is interpretable. The only limitation is the small fraction of results falling into the ATU, which should not be reported as final clinical results.[14] However, since ATUs diminished substantially by 6 hours, we recommend holding any uninterpretable 4-hour results for re-reading at 6 hours. The EUCAST RAST method anticipates this possibility by providing specific ‘uncertain’ categories at each timepoint.[14] Extended incubation beyond 8 hours is generally unnecessary; in our cohort, waiting to 8 hours only converted one additional CTX ATU to an interpretable result. For XDR-prevalent settings where AZM and MEM are the primary therapeutic options, the earlier reporting cutoff, 6 hours for most drugs, is particularly crucial.

Early RAST reporting could have substantial clinical impact on patient management. Bloodstream infections are associated with substantial mortality, and delays in appropriate antimicrobial therapy are associated with increased mortality and poorer clinical outcomes.[22,23] By yielding reliable AST results a day earlier than conventional methods, RAST could guide early de-escalation from broad empiric therapy or prompt escalation if first-line drugs will fail. For instance, in regions with endemic XDR *S. Typhi*, early detection of MEM susceptibility is vital since MEM is essentially the last-resort drug for severe cases.[1,9] Similarly, confirming SXT resistance or AZM susceptibility at 6 hours can influence drug choice, particularly in outpatient settings where oral therapy is preferred. A randomized trial in gram-negative sepsis showed that ‘same-day’ AST reduced mortality and costs compared to next-day AST.[12] While our study did not measure clinical outcomes, the principle is the same, faster microbiology results should translate to better antimicrobial stewardship and improved patient outcomes, especially when coupled

with antimicrobial stewardship teams. The EUCAST RAST method has been implemented with full automation in some laboratories, which can further reduce hands-on time and errors.[24]

Our findings align with other recent evaluations of RAST in diverse settings. The overall CA of 100% (on interpretable results) exceeds or matches published multicenter data. Bianco et al. reported CA of ≥ 99% for gram-negative bacilli in a multicenter Italian study.[15] The complete lack of misclassifications is reassuring; earlier reports had found only rare errors, usually involving ATU cases or borderline zones.[15,16] In practice, this means that in our setting any RAST result that is interpretable should be considered definitive and acted upon clinically. This high degree of accuracy may be partly attributable to the use of a single, well-established laboratory with experienced technicians reading zones consistently.

Several limitations of this study should be acknowledged. First, this was a single-center study focused on *S. Typhi* from a high-prevalence region. Results may differ for other species or in settings with different resistance spectra, different prevalence of XDR, or different patient populations. However, our design using consecutive isolates from a broad range of participants should make the findings broadly applicable to *S. Typhi* in similar high-burden contexts. Second, EUCAST RAST does not have an intermediate category, simplifying our calculations but potentially omitting borderline cases that could be classified differently by other methods. However, the absence of an intermediate category is consistent with EUCAST's approach for many species–antibiotic combinations and reflects the clinical need for definitive guidance on therapy. Third, we did not perform molecular testing to confirm resistance mechanisms or species identification; identification to species was assumed from routine laboratory work using biochemical and serological methods. In practice, correct species identification is required before applying *S. Typhi*-specific RAST breakpoints.[14] Fourth, the study did not include follow-up clinical data to assess whether RAST results influenced therapy or outcomes. Future studies should incorporate clinical outcome measures to evaluate the real-world impact of RAST.

Another consideration is that the EUCAST RAST method requires access to positive blood culture broth and specific equipment for zone measurement. While the method is relatively simple and low-cost compared to molecular susceptibility tests, it does require laboratory personnel trained in disk diffusion and the specific RAST procedure. The method may be most feasible in reference laboratories and larger hospitals with microbiology capacity, but with appropriate training, it could be implemented in regional and district laboratories as well. The use of automated zone readers could further standardize measurement and reduce inter-operator variability.

Future work should validate these findings in additional geographic settings and with larger sample sizes. Studies could also assess the real-world impact of RAST on therapy selection time, length of hospital stay, and clinical outcomes in typhoid fever. As EUCAST continues to refine RAST breakpoints, laboratories should incorporate these tools into routine workflows. Additionally, the role of RAST in settings with rising azithromycin resistance should be evaluated, as azithromycin resistance may be more difficult to detect at early time points because subtle zone differences can limit interpretability.[16]

The potential for RAST to be combined with rapid diagnostic tests for *S. Typhi* should also be explored to create a comprehensive diagnostic algorithm.

The broader implications of our findings extend beyond typhoid fever to other bloodstream infections and antibiotic resistance challenges. The EUCAST RAST method represents a paradigm shift in antimicrobial susceptibility testing, moving from a 24-hour turnaround time to a same-day result. This has implications not only for individual patient care but also for antimicrobial stewardship and infection control. By enabling earlier appropriate therapy and earlier recognition of resistance patterns, RAST could reduce the selection pressure for further resistance and preserve the effectiveness of last-line antibiotics.

CONCLUSION

EUCAST RAST disk diffusion enables highly accurate early antimicrobial susceptibility testing results for *S. Typhi* directly from blood cultures. Reporting RAST at 6 hours could reduce the diagnostic timeline by approximately 1–2 days, a critical improvement in regions with high XDR typhoid incidence. These results support the implementation of EUCAST RAST in routine clinical workflows to enable earlier targeted therapy and improved outcomes in typhoid fever.

Abbreviations

AMR
Antimicrobial Resistance
AST
Antimicrobial Susceptibility Testing
ATCC
American Type Culture Collection
ATU
Area of Technical Uncertainty
AZM
Azithromycin
CA
Categorical Agreement
CTX
Cefotaxime
EUCAST
European Committee on Antimicrobial Susceptibility Testing
IRB
Institutional Review Board
MDR
Multidrug-Resistant

MEM
Meropenem
MIC
Minimum Inhibitory Concentration
RAST
Rapid Antimicrobial Susceptibility Testing
SXT
Trimethoprim/sulfamethoxazole
LUMHS
Liaquat University of Medical Health Sciences
XDR
Extensively Drug-Resistant

Declarations

HUMAN ETHICS AND CONSENT TO PARTICIPATE

This prospective diagnostic accuracy study was approved by the Research Ethics Committee of Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro, Sindh, Pakistan (Reference No. LUMHS/REC/1110). The study utilized anonymized clinical and laboratory data, including positive blood culture specimens that had been originally collected as part of routine patient care. No additional samples were collected for research purposes, and there was no direct interaction between the investigators and study participants. Accordingly, the requirement for informed patient consent was waived by the Research Ethics Committee. The study was conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki, as revised in 2024.

CLINICAL TRIAL NUMBER

Not applicable

AVAILIBLITY OF DATA AND MATERIALS

All data generated during the course of this research study is included in the published article.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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The authors didn’t receive any funding for this work.

Author Contribution

YS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation & Writing – review & editingMAK: Formal analysis, Software, Visualization & Writing – original draftRAM, IDU & FI: Resources & Writing – review & editingSZ: Writing – review & editing

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Data Availability

All data generated during the course of this research study is included in the published article.

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Figures

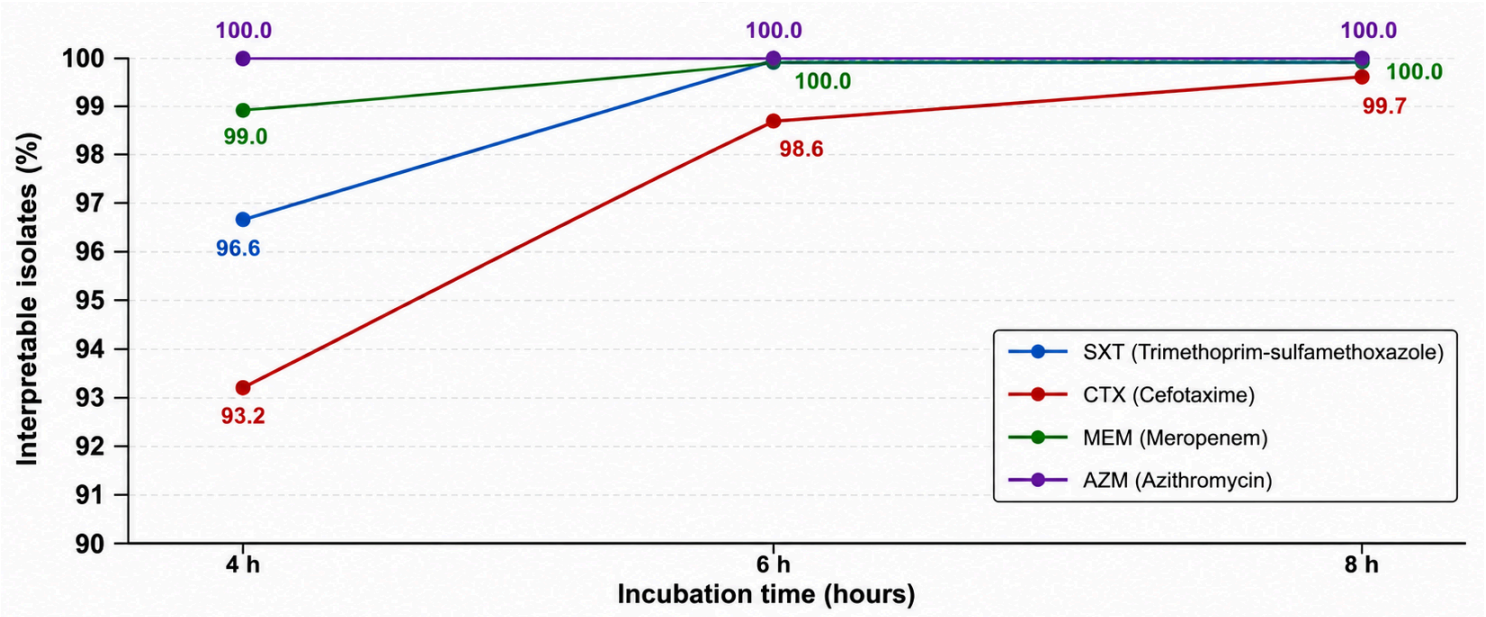


Figure 1

Proportion of interpretable (non-ATU) EUCAST RAST results at 4, 6, and 8 hours.

[**NOTE:**All SXT, MEM, and AZM results were interpretable by 6 hours. CTX interpretability increased from 93.2% at 4 hours to 98.6% at 6 hours and 99.7% at 8 hours. Significant improvement between 4 and 6 hours for SXT (p=0.004) and CTX (P <0.001); no significant improvement between 6 and 8 hours for any antibiotic.]

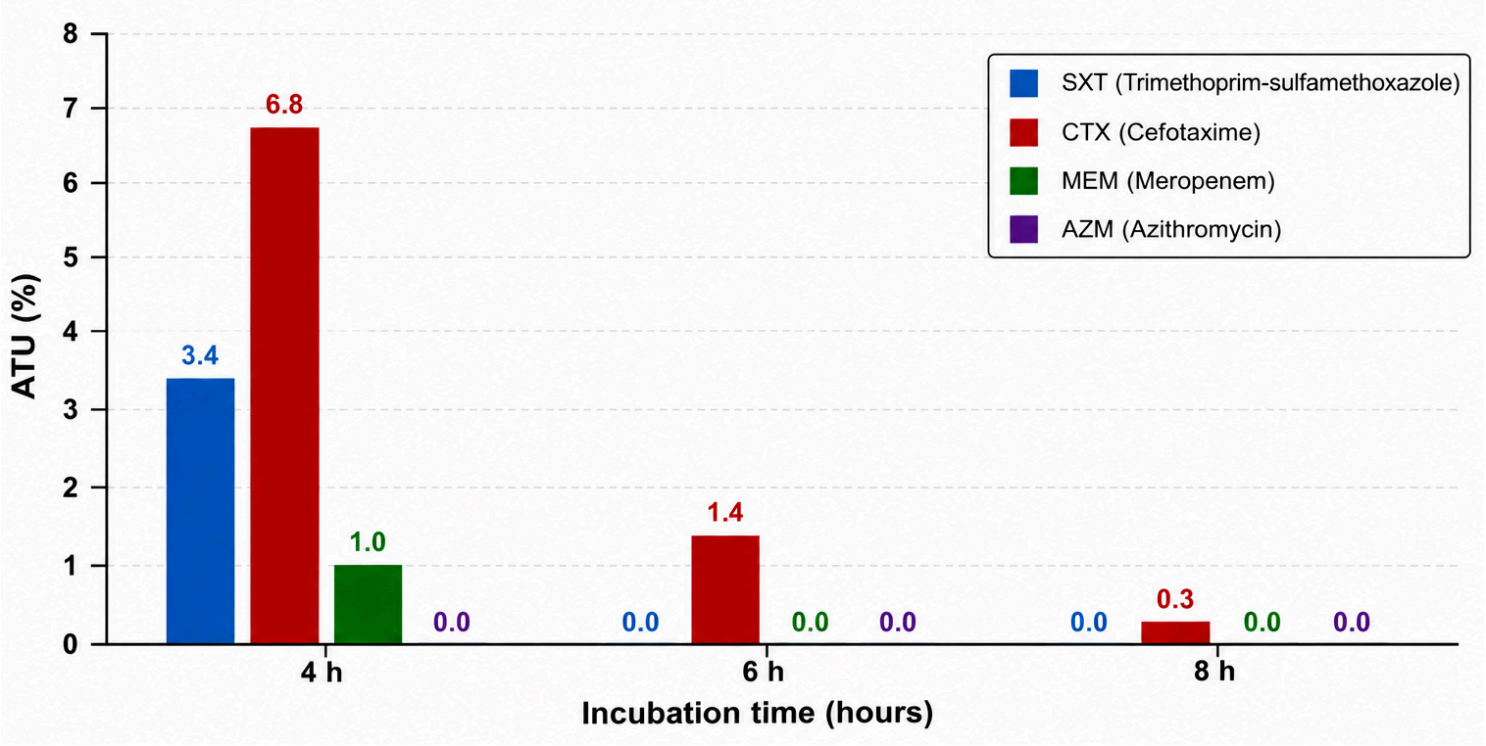


Figure 2

Reduction in the area of Technical Uncertainty (ATU) with increasing incubation time for *Salmonella enterica*serovar *Typhi* isolates (n=294)

[**NOTE:** y-axis represent the percentage of EUCAST RAST results classified within the Area of Technical Uncertainty (ATU) at 4, 6, and 8 hours. ATU rates decreased progressively with increasing incubation time, reaching 0% for SXT, MEM, and AZM by 6 hours. For CTX, ATU decreased from 6.8% at 4 hours to 1.4% at hours and 0.3% at 8 hours.]