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Reagent Costs as Primary Drivers of Biochemical and AST Expenses: Challenging Assumptions of Time-Based Laboratory Efficiency: An Example from Zambia

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Abstract

Microbiological testing underpins effective antimicrobial resistance (AMR) surveillance; however, its financial implications remain poorly characterized in resource-limited settings. This study evaluated the time, labor, and cost components of biochemical and antimicrobial susceptibility testing (AST) in Zambia. Between December 2024 and March 2025, data were collected from 13 laboratories across nine provinces using structured questionnaires. Inputs related to labor and time, reagents, and equipment were analysed using the WHO Laboratory Testing Costing Tool (LTCT) and Julius AI, with pricing guided by the Zambia Public Procurement Act. Ethical approval was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) and the National Health Research Authority (NHRA). A mixed-methods approach was employed to complement quantitative costing with contextual insights. Biochemical tests clustered into two categories based on time requirements: high-intensity tests averaging 78 minutes and rapid tests averaging 13 minutes. Despite this variation, costs ranged from USD 4.25 to 7.99 per test, with rapid tests such as Coagulase being the most expensive due to specialized reagent needs. AST using Mueller Hinton media averaged USD 11.86 per test, with reagents accounting for over 88% of total expenditure. Across all test types, reagent costs dominated, while labor and equipment contributed minimally. Findings reveal a disconnect between test duration and cost, underscoring that efficiency gains are limited primarily by reagent pricing rather than workflow optimization. Strategic procurement, local reagent production, and targeted cost-control measures are essential to sustain affordable microbiological diagnostics in Zambia and similar low-resource settings.

Keywords: *Costs and Cost Analysis, Drug Resistance, Laboratories, World Health Organization, Zambia.*

Introduction

Antimicrobial resistance (AMR) is an urgent global health crisis of the 21st century, endangering the globe to a possible pandemic despite significant medical progress with relation to infectious disease management [1, 2]. With AMR potential to render common infections untreatable, The World Health Organization (WHO) identifies AMR as a paramount global public health concern, that could lead to increased mortality, morbidity,

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and healthcare expenditures globally [2]. A large portion of this AMR burden can be pointed to bacteria featured on the WHO Bacterial Priority Pathogen List (BPPL) [17]. While it is a worldwide challenge, the repercussions of AMR are disproportionately felt in low- and middle-income countries (LMICs) such as Zambia, where constraints in diagnostic capacity, underdeveloped surveillance systems, and inconsistent access to appropriate antimicrobial therapies worsen the problem [4, 5]. In 2019, AMR was linked to an estimated 255,000 deaths in Africa, largely due to factors including imprudent antimicrobial use, suboptimal vaccination coverage, environmental contamination, limited availability of quality diagnostics and medicines, and insufficient public and healthcare worker awareness [5].

Although Zambia's public health system is functional, bacteriology testing capacity remains notably limited. Only a minute 1% of public health laboratories in Zambia are equipped to perform bacteriology testing, severely restricting diagnostic access [6, 7]. Just as in many LMICs, healthcare investments have often prioritized vertical disease programs, such as Human Immunodeficiency Virus (HIV), Tuberculosis (TB), and Malaria, at the expense of strengthening broader diagnostic infrastructure [7]. Consequently, essential microbiological methods, including culture and antimicrobial susceptibility testing (AST), which are cornerstone tools for effective antimicrobial stewardship (AMS), are significantly underutilized. AST services which are crucial for guiding appropriate antibiotic therapy and thereby curbing the emergence of resistance, are only accessible in 55% of second-level hospitals and a mere 23% of third-level hospitals, with virtually no presence at the primary care level [6]. These diagnostic gaps compel empirical antibiotic prescribing, which further accelerates AMR. Furthermore, only 19% of Zambian facilities possess the necessary infrastructure for AMR surveillance, a figure that marginally increases to 34% even in the comparatively better-equipped faith-based hospital facilities [8, 9].

The World Bank estimates that if AMR is not addressed, it could lead to an annual global GDP loss of 3.8% by 2050, accumulating to nearly $100 trillion [10]. Cost analysis within the health sector offers a pivotal tool for quantifying this financial burden and showing the value of investing in diagnostics [30-33]. Such costing data are also instrumental in advocating for the inclusion of AMR diagnostics within essential health service packages [32, 34]. While initial diagnostic expenses may be higher, diagnostic-driven treatment strategies have been shown to improve patient outcomes and minimize unnecessary drug exposure [11]. Despite the undisputed utility of laboratory diagnosis, encompassing microscopy, culture, and AST, in guiding appropriate antimicrobial therapy, particularly in resource-limited settings, there is a scarcity of cost studies specifically exploring these microbiology-specific diagnostics**.** Most existing economic data are inclined to clinical chemistry or newer, often more expensive, diagnostic technologies [12]. This gap is particularly concerning given the proven efficacy of AST in guiding targeted antibiotic use, thereby reducing the reliance on multiple or stepwise broad-spectrum antibiotic regimens that inevitably contribute to the development of AMR [35].

Therefore, this study was designed to assess the time, labor, and cost implications associated with various microbiological biochemical and AST tests in Zambia. By generating a detailed cost analysis, this research aims to provide critical evidence that can influence policy decisions regarding financing for AMR initiatives, ultimately championing the indispensable practice of pre-prescription diagnostic testing to guide antibiotic use.

Materials And Methods

Data were collected between December 2024 and March 2025 from 13 laboratories performing culture and antimicrobial susceptibility testing (AST) across nine provinces in Zambia: Muchinga (2), Lusaka (4), Northwestern (1), Southern (1), Northern (1), Eastern (1), Central (1), Western (1), and Luapula (1). Five provinces were classified as rural and three as urban. A structured questionnaire was administered to capture standardized information on human and material resource inputs required for microbiological testing. The questionnaire collected detailed quantitative and qualitative data [14] on time spent during key steps of bacteriological testing, including media preparation, culture and AST inoculation, and result interpretation. Additional information on Gram stain procedures included average smear reading time and volume of staining reagents used per test. Staff wage data were based on the Ministry of Health full-time salary for a Biomedical Technologist and were consistent across laboratories. Once data saturation was reached, defined as the point at which no new significant information emerged [13], the responses were averaged and summarized. These consolidated values were then entered into the WHO Laboratory Testing Costing Tool (LTCT) [3] to compute unit costs per microbiological test, allowing standardized estimation of direct and indirect costs. Information on testing requirements for microscopy, culture, and AST was obtained from the local laboratory standard operating procedures (SOPs), the American Society for Microbiology [36], and manufacturer protocols from Himedia [16], chosen to align with local supplier quotations. Three Zambian vendors provided detailed cost estimates in Zambian Kwacha, which were converted to U.S. dollars using OANDA [16] exchange rates. All reported costs excluded taxes for consistency. Laboratory reagent and equipment quotations were obtained following the Zambia Public Procurement Act of 2020 [37], which permits simplified bidding (minimum three bidders), limited selection, or single sourcing when only a few suppliers are available. Requests for quotations were sent to eight pre-identified suppliers; three responded for reagents and consumables, and one for equipment. Reliance on a single equipment quotation was legally compliant and reflected the limited supplier landscape [37]. Market prices were used to calculate per-test costs.

Data Analysis

Cost per test for microscopy, culture, biochemical tests, and AST, including tests for WHO Bacterial Priority Pathogen List organisms, was estimated using the WHO LTCT [3]. Key cost components included reagents and consumables, equipment (depreciation and usage), and staff time (converted from minutes to wage costs). Descriptive analyses were conducted using Julius AI [23], an artificial intelligence assistant that selects appropriate large language models to perform statistical analyses and generate code for data processing.

Figure 1 outlines the flow of events during data collection and analysis, highlighting the sequential steps followed in the study.

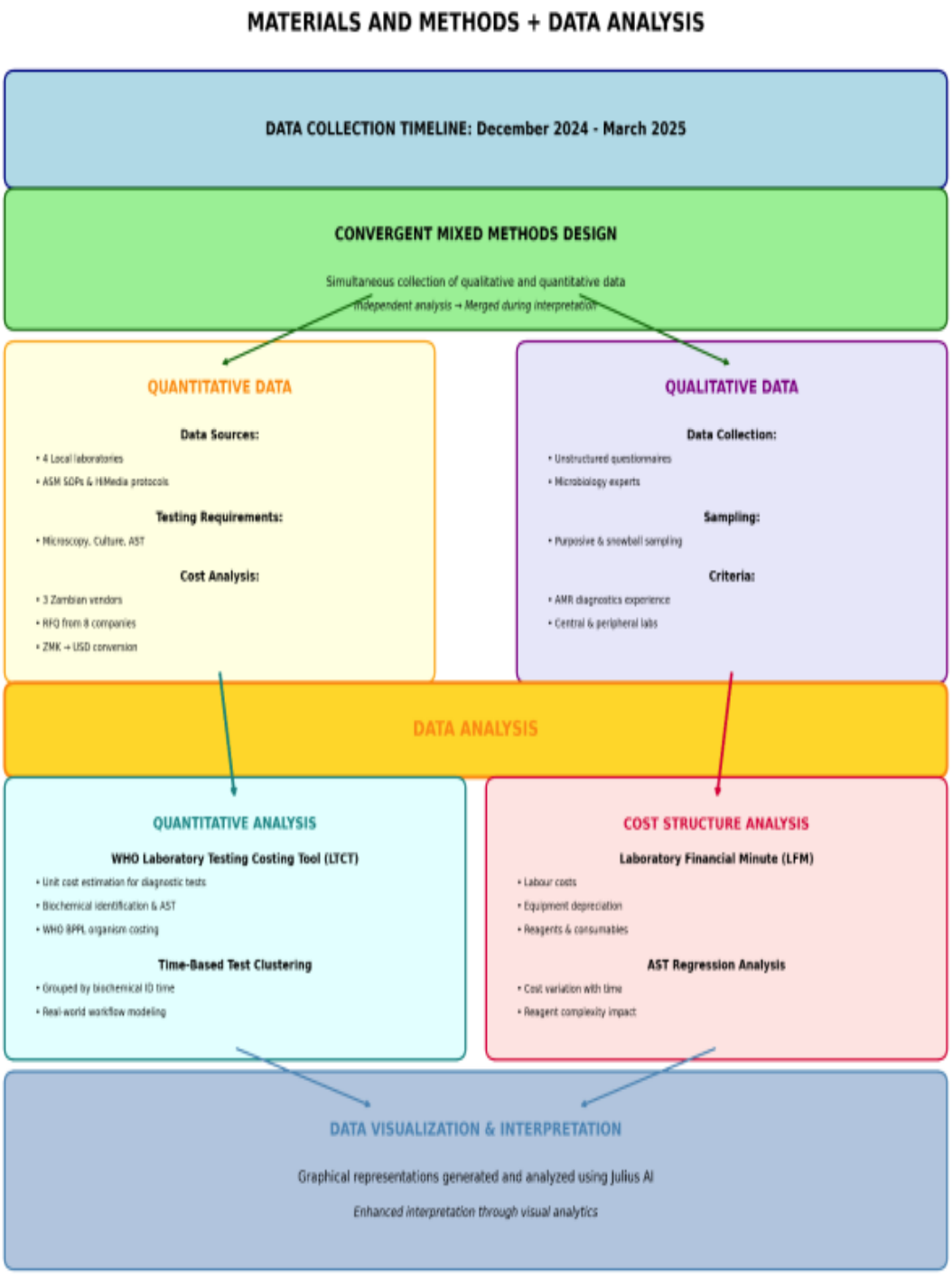

Figure 1. Methods and Materials Illustration

Results

Time Based Clustering of Biochemical Tests

The tests assessed in this study, grouped naturally into two categories based on LFM. High time-intensive tests, including Triple Sugar Iron (TSI), Kligler Iron Agar, SIM Medium, Simmon's Citrate, and Urease, each required approximately 78 minutes. These procedures involve the preparation of solid media, incubation periods, and multi-step interpretation, which significantly increase their time demands. In contrast, a second group of tests, Catalase, Coagulase, and Kovac's Oxidase, demonstrated substantially lower time requirements, averaging just 13 minutes per

test. These "quick tests" are rapid enzymatic or biochemical assays performed using slide or spot methods, with minimal preparation and no incubation, making them far less labor-intensive and more suitable for high-throughput diagnostic settings.

Cost Structure Breakdown of Biochemical Tests Based on LFM

High time-intensive tests, including Triple Sugar Iron (TSI), Kligler Iron Agar (KIA), SIM Medium, Simmon's Citrate, and Urease, had consistent total costs per test ranging from $4.25 to $4.49. Their cost composition was relatively uniform, with reagents and consumables accounting for $3.48–$3.72, fixed equipment costs of $0.35, and staff costs of $0.42, proportional to the longer processing time.

In contrast, the quick tests, Catalase, Coagulase, and Kovac's Oxidase, exhibited greater cost variability, with total costs ranging from $4.30 to $7.99. This variation was primarily due to reagent expenses ($4.23–$7.92), while equipment costs were negligible and staff costs remained minimal ($0.07). Notably, the Coagulase test emerged as the most expensive at $7.99, driven almost entirely by its high reagent cost, despite requiring only 13 minutes and no equipment—highlighting that reagent pricing can outweigh time or equipment in determining cost. Conversely, Simmon's Citrate Medium was the most economical test at $4.25, illustrating the cost efficiency of traditional in-house biochemical media.

The time-cost scatter plot reveals two distinct clusters of diagnostic tests, reinforcing that test duration does not directly predict total cost. While high-time tube tests (e.g., TSI, SIM) exhibit consistent, mid-range pricing due to balanced reagent, staff, and equipment inputs, rapid tests like Coagulase defy expectations by being among the most expensive, driven largely by high reagent costs. This decoupling of time and cost highlights the need for multidimensional analysis when evaluating test efficiency and financial sustainability. In summary, optimizing diagnostic protocols requires considering both workflow efficiency and cost composition, not just turnaround time. Figure 2 illustrates the cost distribution of biochemical tests and outline of laboratory financial minutes versus total cost per test.

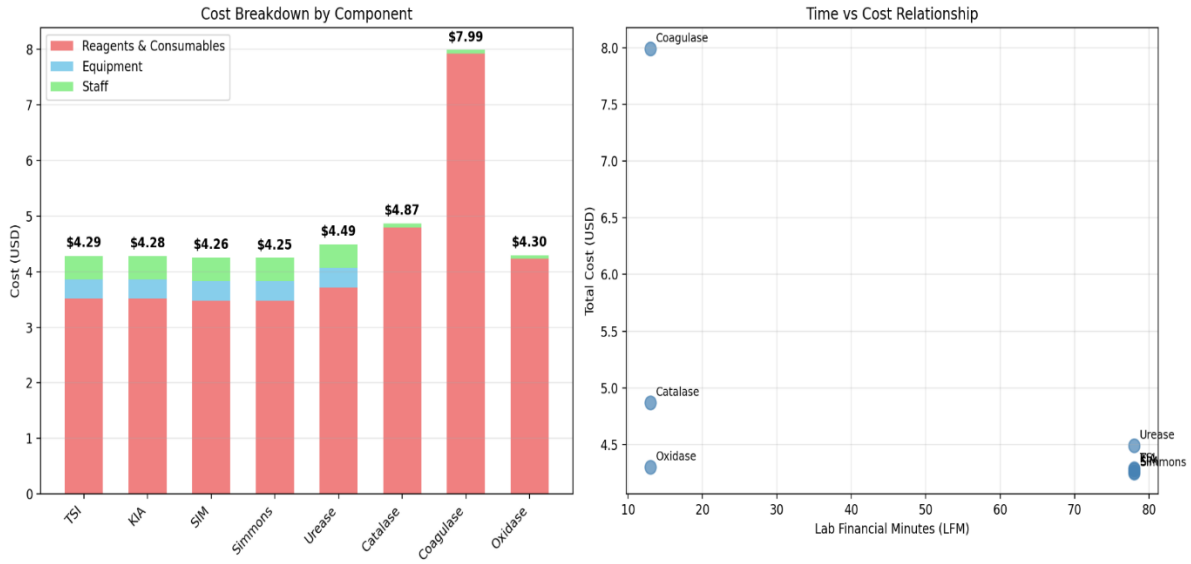

Figure 2. Cost Distribution of Biochemical Tests (left) and Outline of Laboratory Financial Minutes Versus Total cost per test

Regression Analysis: AST total cost as function of time and reagent complexity

The model comparison highlights that reagent costs are the strongest predictor of total antimicrobial susceptibility testing (AST) costs, with a perfect fit ($R^2 = 1.000$, RMSE ≈ $0). Time-based models also predict well ($R^2 = 0.991$), while complexity contributes moderately ($R^2 = 0.658$). A high F-statistic (627.78) confirms statistical significance. The inverse time-cost trend reflects that rapid AST methods are more expensive, despite shorter durations. In practice, laboratories should prioritize reagent cost control for budgeting and procurement. Financial models like Total Cost ≈ $1.14 + 0.90 × Reagent Cost offer accurate, simplified forecasting tools for cost-efficient decision-making and planning. Figure 3 outlines the regression analysis reveals a fascinating cost structure in antimicrobial susceptibility testing that follows predictable mathematical relationships.

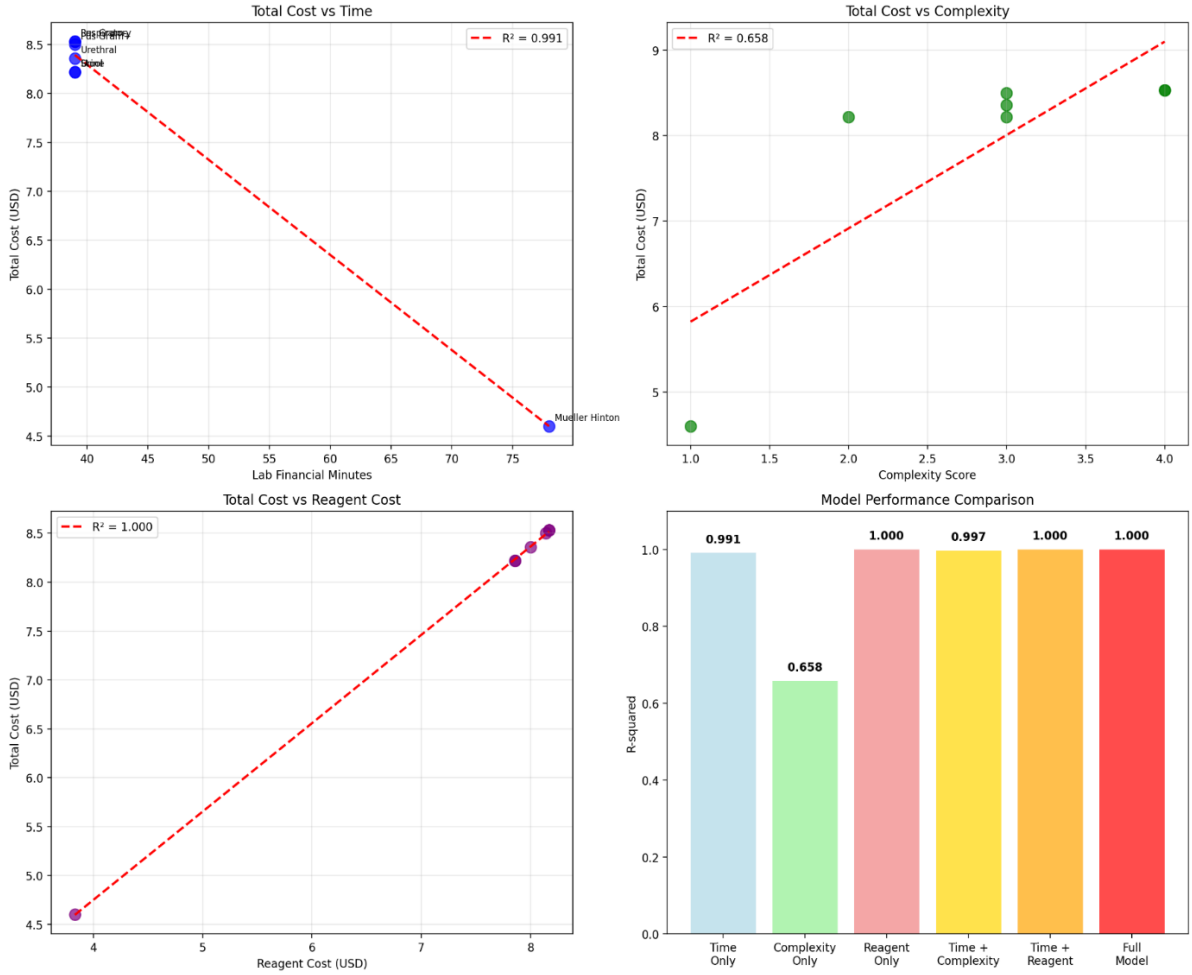

Figure 3. The Regression Analysis Reveals a Fascinating Cost Structure in Antimicrobial Susceptibility Testing that follows Predictable Mathematical Relationships

Discussion

Efficiency Metrics and Workflow Implications

This study's analysis of LFM reveals two distinct temporal clusters of biochemical tests, reflecting underlying methodological and operational differences. High time-intensive tests (e.g., TSI, Kligler Iron Agar, SIM Medium, Simmon's Citrate, and Urease) averaged 78 minutes and aligns with traditional culture-based diagnostic workflows. These procedures involve multiple steps, media preparation, inoculation, incubation, and interpretation, each contributing to extended

turnaround times. Such temporal patterns are consistent with established microbiology workflows that categorize tests by complexity and time investment [24].

Conversely, rapid tests (Catalase, Coagulase, and Kovac's Oxidase) averaging 13 minutes showcase the efficiency of enzymatic spot methods. These rely on immediate biochemical reactions, enabling real-time interpretation and eliminating the need for incubation. The resulting six-fold throughput improvement has meaningful implications for operational efficiency and clinical responsiveness.

Despite the clear differences in LFM, cost analyses revealed a paradoxical decoupling between test duration and total cost. High-intensity tests demonstrated tightly clustered costs ($4.25–$4.49), driven by standardized reagents, consistent equipment usage ($0.35), and proportional labor allocation ($0.42). These findings reflect mature, optimized workflows that benefit from economies of scale in traditional microbiological testing and resonates with the call for Africa to enhance local manufacturing unlike the current situation where 70% and 100% of finished pharmaceutical products, 99% of vaccines, and nearly all medical devices and active pharmaceutical ingredients are imported [28].High cost of reagents can increase inequalities amongst vulnerable communities, driving local manufacturing could potentially reduce the gap in access [20, 29].

In contrast, rapid tests exhibited a wider cost range ($4.30–$7.99), with reagent costs emerging as the dominant variable. This variability underscores the influence of proprietary or commercially prepared reagents in rapid testing protocols. For example, the Coagulase test, despite minimal time and equipment needs, had the highest overall cost ($7.99), largely due to the use of specialized non-in-house reagents. This reinforces the critical role of reagent pricing in determining diagnostic cost structures, independent of complexity or time investment.

These observations have important operational and financial implications. The consistent cost structure of high-LFM tests supports predictable budgeting and centralized procurement strategies. In contrast, the volatile cost profile of rapid tests demands flexible financial planning and emphasizes the importance of strategic reagent sourcing. Furthermore, the weak linear relationship between LFM and total cost, evident in scatterplot analyses, highlights the inadequacy of time alone as a predictor of financial burden. This non-linearity confirms that cost-effectiveness cannot be inferred solely from test duration, a finding consistent with other studies showing that time-efficient tests may be more economical in multitasking environments or settings with part-time staffing [18, 19, 22].

Strategically, laboratories can use these insights to optimize workflow and resource allocation. High-intensity tests may benefit from batch processing [26] and scheduling efficiencies, while rapid tests may require focused cost control strategies such as reagent substitution, supplier negotiation, or selective menu optimization [27]. The identification of Coagulase testing as a cost outlier within the rapid cluster further illustrates the need for test-specific economic evaluations.

Cost Structure and Predictive Modeling

The exceptional predictive power of reagent costs in this analysis underscores the fundamental economics of antimicrobial susceptibility testing (AST), where consumables consistently represent the largest variable cost component. This finding corroborates existing literature in clinical laboratory economics, which consistently identifies reagent expenditure as the primary financial concern for laboratory operations [25]. The near-perfect correlation between reagent costs and total testing expenses suggests that other cost elements, such as

labour, equipment depreciation, and overheads, remain relatively constant across AST methodologies. As such, reagent costs serve as a practical proxy for estimating overall AST costs, simplifying budget forecasting and resource allocation.

One of the most notable findings in the cost breakdown is the remarkably narrow cost range across different AST test types, with only a $0.31 variation. This tight distribution highlights a significant degree of standardization in test materials and procedural workflows, particularly due to the universal use of Mueller Hinton media. The implication for laboratories is substantial, such consistency allows for streamlined procurement, improved budgeting accuracy, and more predictable cost modelling, key factors for national health programs seeking to scale up or decentralize AST services [31].

The analysis further reveals that nearly 90% of AST costs are attributable to reagents and consumables. Equipment and labour contribute minimally to total costs, offering laboratories an opportunity to exert financial control by focusing on reagent-related strategies. Approaches such as bulk purchasing, vendor negotiations, and the use of locally manufactured media can significantly enhance cost efficiency [29]. These strategies align with findings from other studies advocating for localized procurement and inventory management systems as mechanisms for ensuring financial sustainability in laboratory services [12, 21].

However, it is critical to recognize that cost should not be the sole determinant in the decision to introduce or scale up AST. Several studies have demonstrated that despite higher testing costs, the use of faster and more sophisticated diagnostics can result in substantial downstream clinical benefits, including reduced turnaround times, shorter hospital stays, and more targeted antimicrobial use [21, 22]. These outcomes ultimately contribute to broader health system savings and better patient care.

The strong performance of time based cost prediction models ($R^2 = 0.991$) presents an alternative lens for laboratory cost modelling, especially for operational planning. The high correlation likely reflects the integrated demands of AST systems, where longer tests require more reagents and labour. Yet, the slightly lower predictive value compared to reagent cost models suggests that time alone cannot account for variations in reagent quality or procurement dynamics.

In contrast, test complexity demonstrated only moderate predictive power ($R^2 = 0.658$), challenging the assumption that more intricate protocols directly equate to higher costs. This may reflect the efficiency gains from automated systems or inadequacies in the way complexity is currently measured. It also implies that economies of scale may neutralize the expected cost burden of complex tests.

Conclusion

This study demonstrates the natural clustering of biochemical tests into distinct temporal and cost categories, highlighting critical differences in diagnostic methodologies and their operational implications. High-intensity tests exhibited consistent costs and balanced resource utilization, whereas rapid tests showed substantial cost variability, largely driven by reagent prices. The observed decoupling between test duration and total cost challenges conventional assumptions about time-based efficiency and emphasizes the dominant influence of reagents on overall expenses. These findings provide a framework for optimizing test selection, resource allocation, and workflow planning in clinical laboratories.

The study has several limitations. The cost analysis focused primarily on direct expenses and did not fully capture indirect or hidden costs such as maintenance, infrastructure, training, or regulatory compliance.

Additionally, cost models did not account for bulk purchasing agreements, supplier-specific pricing, or regional market fluctuations. Time data were based on standard laboratory minutes and may not reflect real-world variations in technician performance or workload distribution.

Future research should integrate clinical outcomes, including patient recovery, antimicrobial stewardship metrics, and hospital length of stay, to develop comprehensive assessments of diagnostic value. Multi-centre and cross-regional studies are recommended to validate findings across diverse laboratory contexts. Exploration of emerging technologies, such as molecular diagnostics and AI-enhanced systems, will be important for forecasting future cost structures and guiding investment in laboratory infrastructure and innovation.

Based on these findings, several strategic recommendations emerge. Laboratories should prioritize reagent cost management through bulk purchasing, local sourcing, and supplier negotiation. Monitoring cost-per-test, particularly for rapid diagnostics with high variability, will enhance financial control. Workflow optimization should consider separate processing tracks for high-intensity and rapid tests, and in-house media preparation can reduce dependence on commercial products. Test selection should align with clinical urgency, using rapid tests for time-sensitive cases and high-intensity tests for routine procedures. Staff training should emphasize time-cost dynamics to improve task efficiency. At the policy level, the results can inform the design and scale-up of antimicrobial susceptibility testing programs, supporting evidence-based investment and procurement decisions. Replicating similar cost-time analyses in multiple laboratory settings will strengthen generalizability and promote more efficient, sustainable, and data-driven diagnostic services.

Ethical Consideration

Ethical approval was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC), 6016-2024 and the National Health Research Authority (NHRA), approval number NHRA-1802/18/12/2024. Informed consent was secured from all participants.

Data Availability

The datasets generated and analysed during this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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Author Contribution

All authors contributed to the study in specific ways as outlined below:

1. **Lutinala Nachilembo Nalomba:** Lutinala led the conceptualization and design of the study and was primarily responsible for developing the research proposal. She took the lead in reviewing the data analysis and drafting the report and also spearheaded the writing and critical revision of the manuscript.
2. **Mox Kalumbi:** Mox played a key role in developing the data collection tool and provided practical insights into on-site

microbiology practices. He participated in data collection and contributed to reviewing both the proposal and the manuscript.

3. **Baron Yankonde:** Baron contributed to the development of the data collection tool and offered practical input on microbiology practices during fieldwork. He participated in data collection, reviewed the proposal and manuscript, and supported the compilation of technical requirements and distribution of vendor quotation requests.
4. **Pascalina Chanda-Kapata:** Pascalina contributed to the study's conceptualization and design and supported the development of the proposal. She also participated in reviewing the data analysis, report, and manuscript.

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