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Assessing Feasibility of Point-of-Care Antibiotics Susceptibility Testing Technologies for Mitigating Access Gaps for Peripheral Communities

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Abstract

Background: Antibiotic resistance (AMR) is unquestionably one of the biggest issues facing developing nations today. Almost half of all antibiotic regimens begin with the incorrect drugs and without an adequate diagnosis of the microorganism that causes AMR. Therefore, there is a growing demand for methods and diagnostics that enable rapid antimicrobial susceptibility testing (AST). Point Of Care Test (POCT) would potentially lead to improved access, more effective treatment and faster resolution of infections and this paper evaluates performance of one such technology.

Methods: We evaluated the performance of a new POCT "RightBiotic" for the rapid bacterial identification and prediction of AST. This study was conducted in two pre-implementation assessment rounds to validate the functionality and efficacy of this technology and results compared with those achieved through conventional culture methods.

Results: Based on our results, RightBiotic enabled bacterial identification and AST results within 4 hours when compared with conventional testing methods based on VITEK 2. Out of the 5 isolates identified by conventional method, only 3 isolates matched with the POCT results while AST results for these 3 isolates were not 100% matched. By reducing the typical timeframe for susceptibility testing from 2–3 days to 4 hours, the POCT phenotypic AST can provide critical information to clinicians prior to the administration of antibiotic therapy, can potentially enhance the efficacy of isolate identification and susceptibility results.

Conclusion: POCT in clinical samples holds great promise for improving healthcare delivery and accessibility, especially in underserved areas, to enable better-targeted antibiotic use necessary for antibiotic stewardship. Therefore, systematic evaluation of such POC testing and building cost-effectiveness models is an urgent priority for research and strengthening clinical practice.

Keywords: Antimicrobial Resistance, Point Of Care Testing, Antimicrobial Susceptibility Testing, Community Settings, Clinical Microbiology.

Introduction

Antimicrobial resistance (AMR) is a significant public health issue that is posing a danger to the effective prevention and treatment of a growing array of infections [1, 2]. This condition is potentially responsible for clinical infection treatment failures in both community and hospital settings. Antibiotic therapy against pathogens is generally administered empirically, based on Antibiotic Susceptibility Test (AST) results, which is a laboratory procedure used to determine which antibiotics are effective against a specific bacterial infection. The purpose of testing is to identify any potential drug resistance in common pathogens and to confirm drug susceptibility for specific illnesses [3]. Broth microdilution and quick automated instrument procedures that employ materials that are sold in markets are the two most popular testing techniques. The disc diffusion and gradient diffusion methods are manual techniques that provide flexibility and potential financial savings. Empirical antibiotic prescription without AST reports often results in unsuccessful therapy, recurrence, and development of AMR [4].

Limited access to AST related diagnostic infrastructure is a particular and growing challenge in low and middle-income countries (LMICs). This challenge is heightened particularly for those people who are living in rural

community settings, far away from tertiary health facilities, where microbiology testing facilities are currently available within the public system [5]. This leads to the important research and practical problem of how AST access can be bought closer to communities to enable more cost-effective and rapid detection of infections. In this regard, point of care testing (POCT) provides the potential to help mitigate, at least to some extent, this gap. Point-of-care (PoC) testing is defined as pathology testing carried out (on small, portable medical devices) in a clinical setting (like a primary care-based medical service) at the time of patient consultation, producing a quick result (typically within minutes or hours) that enables prompt clinical action for patient care [6]. However, little systematic empirical and research evidence exists on the feasibility of using POCTs for clinical practice in community-based health settings. To contribute to this research gap, in this paper, we evaluate the performance of a new POCT for the rapid identification of bacteria and the prediction of antimicrobial susceptibility in clinical microbiological samples. The purpose of this study is to examine the potential of adopting this technology for supporting clinical practice in rural community settings, specifically within the context of the public health system in India.

Relevant literature

Antimicrobial misuse is common in LMICs, which acts as a significant driver of AMR. LMICs may have the fewest assets and data on the epidemiology and burden of AMR, despite having the greatest loads of infectious illnesses [7]. The reason of AMR in LMICs is due to inadequate laboratory and communications infrastructures, scarce resources, a lack of qualified and trained people, and several socioeconomic and behavioural factors, the obstacles in low-income settings are tremendous [2]. The use of antibiotics for human consumption rose by 35% between 2000 and 2010, especially in LMICs, where widespread antibiotic usage both inside and outside the established healthcare system is contributing to the rise in resistance [8].

Antibiotic resistance and use in the community are influenced by various factors like; overprescription and lack of prescription without AST by physician, patient demand, self-medication, incomplete treatment, inadequate diagnostic tools, and other socio-economic factors [9]. According to a comprehensive evaluation of 34 studies investigating the variables influencing antimicrobial self-medication in LMICs, 39% of respondents self-medicate. The primary sources of self-medication were identified as pharmacies, drug stores, over-the-counter (OTC) purchases, and leftover or borrowed medications [10]. Another analysis of 15 studies that looked at variables influencing antimicrobial self-medication in LMICs reported that self-medication prevalence varied widely, from 8% to 93% [11]. The accessibility, affordability, the condition of healthcare facilities, prior successful use of antibiotics, educational levels, and health-seeking behaviour were identified as key sociocultural determinants of health that affect self-medication in LMICs [10]. Numerous studies indicate that a significant portion of antibiotic usage takes place in the community, where they are easily accessible and may be purchased without a prescription. Over 50% of antibiotic prescriptions are inappropriate globally, and in LMICs, two-thirds of antibiotics available at pharmacies are sold over-the-counter [12]. Therefore, one of the main issues for the improper and excessive use of antibiotics in the community is self-medication and easy access to antibiotics without a prescription.

Another factor is the prescrition of antibiotics by doctors in hospitals and general practitioners (GPs) without appropriate AST report or diagnostics. Clinicians work directly with patients in primary care settings and serve as the public's initial point of contact with healthcare providers [13]. However, without rapid tests, it is particularly challenging to distinguish between a bacterial and viral infection based only on the patient's medical record and

physical examination [14]. Nearly 50% of the world's population is thought to have limited or no access to diagnostic testing. For LMICs, where delays in diagnosis are a significant source of illness and mortality, this is a significant problem. Many of occasions, tests are simply not accessible, and even when they are, patients must pay for access to private clinics out of pocket. However, individuals with limited resources frequently opt to go directly for receiving medications when given the choice because diagnostic testing frequently costs more than low-cost generic antibiotics [15].

The lack of innovative, accessible, and inexpensive diagnostics makes combating antibiotic resistance difficult. Inadequate and unequal access to diagnostics can result in misdiagnosed infections or delays in diagnosis, both of which can be damaging to the patient and make long-term treatment more difficult and expensive [16]. Together, this indicates that community-based doctors are now having difficulty making precise, evidence-based diagnoses and providing targeted antibiotics. Introducing POCT diagnostics in community health clinics is one potential solution to this issue. While vendors advertise a wide range of PoC diagnostics, none have yet demonstrated clinical efficacy in the public health system.

Deploying POCT based diagnostic tools for detecting AMR in clinical settings represents a fruitful and important area of future research. A quick, reliable, and reasonably priced AST is necessary since about 50% of antibiotic treatments begin with the incorrect drugs and without a correct pathogen identification [17]. Antimicrobial therapy is currently advised to be guided by validated growth-based AST in accordance with EUCAST (European Committee on Antimicrobial Susceptibility Testing) or CLSI (Clinical Laboratory Standards Institute) guidelines [18]. If samples are sent to a centralised diagnostic laboratory for conventional "gold standard" testing, it may take several days for the results of pathogen isolation, identification, and antibiotic resistance detection to reach the indenting clinician, making it impossible to provide patient care when it is most necessary. The use of POCT devices could reduce the need to take a specimen to a lab and wait days for findings by possibly examining entire blood, urine, and other bodily fluids and making test results quickly available to the patient at the point of care. POCT can aid in the incorporation of clinical patient care processes since it allows doctors to ask for a test, obtain findings, and implement clinical action within the same visit [19]. This offers the chance for quicker treatment intervention and the possibility for better patient results.

In general, there are three "classes" of POC diagnostics. i) PoC tests based on biomarkers; biomarkers are biological substances (other than antigens) that exist naturally in living things and may be utilized to identify a sick condition when abnormalities in their concentrations occur from the physiologically normal value; ii) molecular (nucleic acid-based) PoC tests; In order to diagnose specific diseases, nucleic acid-based PoC tests look for the presence of the pathogen's genetic material (DNA or RNA); iii) immunological PoC tests look for the presence of an antigen (or its molecular counterpart) or an antibody that is produced as an immune response to an infection [20]. In addition, gene sequencing-based POCT is a diagnostic approach that involves the use of gene sequencing techniques to analyze genetic material (DNA or RNA) from a patient's sample [21]. CRISPR-based diagnostics have recently come to light as promising technologies with the potential to completely transform the molecular diagnostics industry. These platforms might democratise access to illness diagnostics since they are affordable, straightforward, and do not need the use of specialised apparatus. Drug-resistant genes in bacteria can be found using CRISPER-Cas, making it easier to choose the right course of therapy and lowering antibiotic misuse [22].However, POCTs come with their limitations as well, as they currently tend to be more expensive than traditional laboratory testing. In terms of performance, not all POCTs are produced equal; some exhibit lesser

sensitivity and specificity than others. It's critical to understand how these test parameters may affect findings that are false-positive or false-negative and the necessity for confirmation testing [23].The full potential of POC testing cannot be realized until these limitations are addressed and resolved and to reduce the time and access constraints of current 'gold standard' antibiotic resistance detection strategies [24].

In India, some strides have been taken to implement POCT in healthcare settings, although it is still in its early stages [24–27]. The medical facilities in India represent a very complex scenario as the country accommodates the most diverse population in the world. Because of significant existing health inequities, healthcare professionals operate under conditions of tremendous care burden and resource constraints that causes inefficiencies and sub-optimal treatment affecting the lives of millions, particularly those who are under privileged. India is a resource-limited country and thus the utility of POC diagnostic devices on broad-scale holds significant promise to combat AMR. This paper contributes to the challenge of understanding how to realize this potential in clinical practice.

To convince policy makers on the value of adopting POCTs, the following criteria are important: i) accuracy of results to ensure correct diagnosis; ii) cost-effectiveness, which can ensure sustainable use by the public health system and is affordable by poor patients; iii) supporting infrastructure within a public community system. In terms of accuracy, POCT technologies need to undergo rigorous clinical validation and meet regulatory standards for accuracy and provide real-time test results, reducing the risk of misdiagnosis or delayed treatment and improving patient outcomes. POCTs should be cost saving by reducing unnecessary hospital admissions, optimizing antibiotic prescriptions, and the need for expensive and sometimes redundant diagnostic procedures, minimizing staff time and saving lab equipment costs. In this study, we have used the above criterion to examine our empirical case. The benefits of a POCT cannot be overlooked but given the nascent phase of its use in India, it is urgent and necessary to validate its cost-effectiveness, particularly in the context of community settings [28]. Only when such evidence can be demonstrated will policymakers be encouraged to adopt this technology in clinical settings.

In the present study both conventional and POCT (using RightBiotic) methodologies were applied for the identification and susceptibility testing of isolates isolated from clinical samples (urine, pus, and blood) in a laboratory at the Postgraduate Institute of Medical Education and Research Chandigarh (PGIMER), and the results were compared. RightBiotic is a platform that provides rapid identification and AST of bacterial pathogens causing infections in the human body. This technology holds the promise of being deployed in clinical settings, provided its efficacy can be established. Examining this is the aim of this paper.

Material and Methods

RightBiotic technology has been developed by a team of researchers at the Birla Institute of Technology and Sciences (BITS), Hyderabad which can identify disease causing bacteria in blood and urine at a faster rate than conventional methods. The technology was tested within the ambit of a research project called "Equity AMR-Analysis of the role of health inequities in Antimicrobial (AMR) policy and practice". While this project covered domains of surveillance, prescribing and diagnosing, this paper focuses on the diagnostics component. The analysis concerned examining its potential for suggesting possible antibiotics for treating the diseases caused by the identified bacteria, faster than by conventional methods which typically takes 48-72 hours. We also examined if the technology can not only help to identify the disease causing bacteria, but also suggest suitable antibiotic drugs to be used to kill those disease causing bacteria. As we were concerned with cost issues, we examined both the cost of the equipment and its everyday operational cost. The equipment developed on a semi-automated

desktop device cost INR 0.45 million. Operationally, it required antibiotic panels which can be customized based on the hospital needs, without external accessories. Everything required for its operation was included in the kit, which cost about INR 550 for testing a urine and pus sample and INR 800 for a blood sample. A panel of 14 antibiotics costed around INR 300.The estimated unit cost of testing of urine or a pus sample was INR 550 and INR 1500 for blood sample. In comparison, the estimated cost of testing these clinical samples using conventional methods was INR 300.

Study design

The study was performed in two pre-implementation assessment rounds conducted at the Enteric laboratory of the Postgraduate Institute of Medical Education and Research Chandigarh (PGIMER) Chandigarh, which is a tertiary care hospital in North India. The study was undertaken under the guidance of an eminent and senior microbiologist. The lab provides diagnostic (conventional as well as molecular), surveillance, referral services, and has extensive prior research in the field of diarrhea, foodborne infections, and urinary tract infections with a special focus on epidemiology and drug resistance. The lab is currently engaged in constantly monitoring AMR in Entero and Uro-pathogens at the community and hospital levels by phenotypic and molecular assays.

Round 1 assessment

This study was conducted in two pre-implementation assessment rounds to validate the functionality and efficacy of the RightBiotic prior to its potential deployment in the field, which were two working community-based labs of PGIMER in Kangra and Baddi, within the public health system in the state of Himachal Pradesh in Northern India. The central PGIMER microbiology lab was designated for the demonstration and testing of the technology. The first round, spanning two days, primarily focused on demonstrating the capabilities of the technology, including required operational procedures and protocols, to the staff from the two pilot labs. Following the presentation, a practical session was conducted by the RightBiotic technical team, who received three samples each of urine, pus, and blood for testing, which were simultaneously also tested in the PGIMER lab for enabling a comparative analysis of the results. The results for the urine and pus samples were obtained on the same day, as they required a 4-hour incubation period, while the blood sample necessitated a longer incubation period of 14-18 hours, with the results obtained on the second day.For the blood sample, the results did not match. Following the testing, a hands-on training session was conducted for the PGIMER staff to familiarize them with the operation of the POC.

While the testing process proceeded relatively smoothly, several challenges were encountered. Firstly, there were delays in obtaining the samples on the first day due to timing constraints related to sample collection at the laboratory. The processing of samples using RightBiotic was found to be rather laborious for the PGI staff, who were well experienced to the conventional methods. This was attributed to the multiple manual steps involved in sample processing, from preparing the sample media to placing the sample in the machine. Moreover, only three samples could be processed simultaneously, which was inadequate for a clinical setting which receives high numbers of samples every day. The RightBiotic team had five test kits for this testing process, but unfortunately, one of the kits was accidentally broken during the hands-on session and one sample was found to be contaminated on the first day. Following the testing, the results were promptly shared with the microbiologist who recommended conducting a more substantial number of sample tests, based on the understanding that assessing the efficacy and performance of POC with only three samples was insufficient. Consequently, at a later date, a second round of testing was carried out.

Round 2 assessment

In this round, a total of 17 clinical samples (14 urine, 2 pus, and 1 blood) were tested by RightBiotic and also by conventional culture methods. For testing by RightBiotic, the manufacturer's instructions (Fig. 1) was complied with.

Processing of clinical samples by conventional method:

The urine samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar medium. Inoculated agar plates were incubated initially aerobically at 37°C for 24 hours and finally for 48 hours, and the plates were examined for pure growth. A growth of $\geq 10^5$ colony-forming units/ml was considered significant. Cultures with more than two colonies were considered as contaminants and such samples were discarded.

Pus samples were processed for gram staining and culturing. The samples were aseptically inoculated on blood agar (with 5% sheep blood) and MacConkey agar plates, incubated aerobically at 35°C–37°C for 24–48 hours. For the conventional blood culture method, blood culture for bacterial infections was carried out in two bottles containing 50 ml each of tryptone soy broth and bile broth. After removing the kraft paper, blood culture bottles were inoculated. These were incubated at 37ºC and examined daily for 7 days for evidence of growth, which was indicated by turbidity, hemolysis, gas production, discrete colonies, or a combination of these. The broth from the positive bottle was sub cultured onto 5% sheep blood agar and MacConkey plates incubated similarly as above for the pus samples.

Bacterial identification by MALDI-TOF MS:

For identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), a single pure colony of the bacterium was directly spotted on the MALDI-TOF MS sample slide. In brief, a pure bacterial colony was smeared onto a steel target plate with a wood toothpick, and a 70% formic acid solution was added to lyse the bacterial cells. Matrix α-cyano-4-hydroxycinnamic acid was added to the sample and allowed to air dry for 5 minutes. The slide was then inserted into the MALDI-TOF MS machine for laser desorption/ionization. The mass spectrum data was then compared with the reference database to identify the bacteria. According to the manufacturer, a score of ≥2.0 indicates reliable identification at the species level, while a score between 1.7 and 1.99 indicated a reliable identification at the genus level, and a score below 1.7 indicates a no reliable identification (NRI).

Antibiotic sensitivity testing

AST was carried out with the VITEK 2[1] system and RightBiotic system for the following antibiotics; amikacin, ceftazidime, cefepime, piperacillin, tazobactum, imipenem, ciprofloxacin, gentamicin, amoxycillin, ofloxacin, ceftriaxone**,** cefuroxime**,** tobramycin, levofloxacin, ceftazolin, cefotaxime**,** cefoperazone-sulbactum, ertapenm**,** meropenem, tigecyclin, colistin, nalidixic acid, cotrimoxazole, nitrofurantoin accordingto the manufacturer's instructions.

[1] VITEK 2 is a fully automated system that performs bacterial identification and antibiotic susceptibility testing. https://www.biomerieux-usa.com.

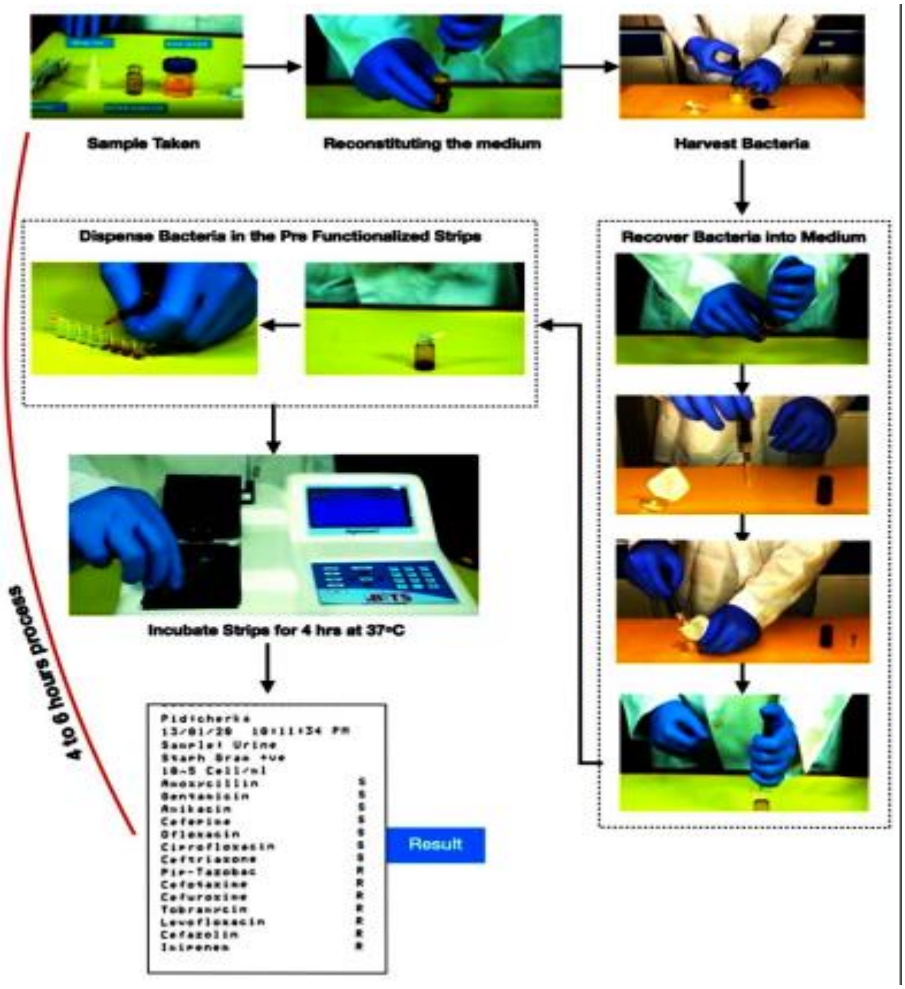

Fig 1. Instrument schematic and AST workflow.

Results

Bacterial identification

A total of 17 clinical samples were analyzed in this study, including urine (n=14), pus (n=2) and blood (n=1). Table 1 shows the sample types and identification of pathotypes by the conventional culture method (gold standard) and by RightBiotic system. RightBiotic demonstrated sensitivity (58%) and specificity (63%) in bacterial identification and predicting of antibiotic susceptibility respectively. Using the conventional method, 5 isolates were identified from 17 clinical samples whereas 10 isolates were identified using RightBiotic. By comparing both methods, 47% (8/17) of sample identifications were matched. Out of these 5 samples were sterile and 3 were identified as *E. coli* (1 pus and 2 urine) as shown in Table 1.In RightBiotic, bacteria were detected in clinical samples (S10, S12, S13, S15, and S16) whereas these samples were identified as sterile by conventional culture methods as shown in Tables 2 and 3.

Table 1: Sample types and identification of isolates by conventional culture method and by RightBiotic.

Sr. No.	Sample ID	Sample Type	Conventional culture method	RightBiotic Identification	Results
1	S1	Blood	*Pseudomonas aeruginosa*	*E. coli*	Major error
2	S2	Pus	*E. coli*	*E. coli*	Correct identification
3	S3	Pus	*Klebsiella pneumoniae*	*E. coli*	Major error
4	S4	Urine	*E. coli*	*E. coli*	Correct identification
5	S5	Urine	Yeast	Sterile	Major error
6	S6	Urine	Sterile	Sterile	Correct identification
7	S7	Urine	Sterile	Sterile	Correct identification
8	S8	Urine	Sterile	*Sterile*	Correct identification
9	S9	Urine	Sterile	*E. coli*	Major error
10	S10	Urine	Gross contamination	*Klebsiella*	Major error
11	S11	Urine	Sterile	*Sterile*	Correct identification
12	S12	Urine	Sterile	*E. coli*	Major error
13	S13	Urine	Sterile	*Klebsiella*	Major error
14	S14	Urine	Sterile	Sterile	Correct identification
15	S15	Urine	Sterile	*Klebsiella*	Major error
16	S16	Urine	Sterile	*Staphylococcus*	Major error

17	S17	Urine	*E. coli*	*E. coli*	Correct identification

Comparison of VITEK 2 and RightBiotic AST test results

Table 2 showing the bacterial identification and AST results by using VITEK 2 system and Table 3 with results from RightBiotic. On comparison, out of 17 clinical samples 8 samples were matched in both methods, out of these, bacteria were isolated in 3 samples (S2, S4, and S17). AST results were interpreted as showing major and minor errors on comparison. Major Error was defined as "when reference method categorized as susceptible but the RightBiotic method categorized it as resistant (falsely resistant). They were calculated by using the number of susceptible isolates as the denominator". Minor error was defined as "when the reference method categorized an organism as intermediate susceptible but RightBiotic categorized it as resistant or susceptible. The percentage of minor errors was calculated by using the total number of organisms tested as the denominator". A major error was found for sample S2, for Gentamicin where it was sensitive in the VITEK 2 system and resistant in RightBiotic. For sample S4, a major error was seen for antibiotics Amikacin and Imipenem, which were sensitive in the VITEK 2 system but identified as resistant in RightBiotic. For sample S17, a minor error was observed for Gentamicin and Amikacin, where in VITEK 2 these antibiotics were sensitive and resistant respectively, whereas shown as intermediate in RightBiotic (refer to Tables 2 and 3).

Table 2. Bacterial identification and Antibiotic Susceptibility Testing results by using VITEK 2 system and RightBiotic POC system.

				Name of Antibiotics																						
Sr. No.	**Sample ID**	**Sample Type**	**Culture**	**AK**	**CAZ**	**CPM**	**PIT**	**IE**	**CIP**	**GEN**	**AXM**	**OF**	**CTR**	**CXM**	**TOB**	**LE**	**CFZ**	**CTX**	**CFS**	**ETP**	**MRP**	**TIG**	**CL**	**NA**	**COT**	**NIT**
1	S1	Blood-Conventional	*Pseudomonas aeruginosa*	S	S	S	S	S	S	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		RightBiotic	*E. coli*	S	NA	S	R	S	S	R	I	S	S	R	S	S	R	R	NA	NA	NA	NA	NA	NA	NA	NA
2	S2	Pus-Conventional	*E. coli*	S	NA	R	S	S	R	S	NA	NA	NA	NA	NA	NA	NA	R	S	S	S	S	I	NA	NA	NA
		RightBiotic	*E. coli*	S	NA	R	S	I	I	R	R	I	R	R	S	I	R	R	NA	NA	NA	NA	NA	NA	NA	NA
3	S3	Pus-Conventional	*Klebsiella pneumoniae*	S	NA	S	S	S	I	S	NA	NA	NA	NA	NA	NA	NA	R	S	S	S	S	I	NA	NA	NA
		RightBiotic	*E. coli*	R	NA	R	R	R	R	I	I	R	R	R	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA
4	S4	Urine-Conventional	*E. coli*	S	NA	NA	S	S	R	R	NA	NA	NA	NA	NA	NA	NA	R	S	S	S	NA	IS	R	R	S

		RightBiotic	*E. coli*	R	NA	NA	S	R	R	R	R	R	R	R	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA
5	S10	Urine	Gross contamination	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		RightBiotic	*Klebsiella a*	S	NA	S	S	S	S	R	R	R	S	S	I	S	R	I	NA	NA	NA	NA	NA	NA	NA	NA
6	S12	Urine-Conventional	Sterile	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		RightBiotic	*E. coli*	R	NA	R	R	R	R	R	R	R	R	R	I	R	I	R	NA	NA	NA	NA	NA	NA	NA	NA
7	S13	Urine-Conventional	Sterile	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		RightBiotic	*Klebsiella a*	R	NA	R	R	R	R	R	R	R	R	R	I	R	I	R	NA	NA	NA	NA	NA	NA	NA	NA
8	S15	Urine-Conventional	Sterile	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		RightBiotic	*Klebsiella a*	I	NA	S	R	R	S	S	R	I	I	S	R	I	I	I	NA	NA	NA	NA	NA	NA	NA	NA
9	S16	Urine-Conventional	Sterile	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		RightBiotic	*Staphylococcus*	I	NA	R	R	R	R	I	I	R	R	I	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA

10	S17	Urine-Conventional	*E. coli*	S	NA	NA	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	R	R	NA	R	NA	IS	NA	R	R
		RightBiotic	*E. coli*	I	NA	R	R	R	R	I	I	R	R	I	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA

Here, S- Sensitive, I- Intermediate, R- Resistance

AK-Amikacin, CAZ- Ceftazidime, CPM- Cefepime, PIT- PiperacillinTazobactum, IE- Imipenem, CIP-Ciprofloxacin, GEN- Gentamicin, AXM- Amoxycillin, OF- Ofloxacin, CTR- Ceftriaxone, CXM- Cefuroxime, TOB- Tobramycin, LE- Levofloxacin, CFZ- Ceftazolin, CTX- Cefotaxime, CFS- CefoperazoneSulbactum, ETP-Ertapenem, MRP- Meropenem, TIG-, Tigecyclin, CL- Colistin, NA- Nalidixic Acid, COT- Cotrimoxazole, NIT-Nitrofurantoin

DISCUSSION

Our comparative analysis showed that 47% (8/17) of sample identification matched and out of which 5 samples were sterile and three were infected. However, it was lower than a study conducted by Maris et al., where they tested 106 whole blood, plasma samples, 96 urine, 28 sputum and 20 pus samples from 112 patients using Active Melioidosis Detect™ (AMD) POC, of whom 26 (23.2%) were culture-positive for B. *pseudomallei* [29]. The AMD POC sensitivity and specificity were 65.4 and 87.2%, respectively [29]. In an another study by Schot et al., who tested six POCT urine analysers: Uryxxon Relax (Macherey Nagel), Urisys 1100 (Roche), Clinitek Status (Siemens), Aution 11 (Menarini), Aution Micro (Menarini) and Urilyzer (Analyticon). Analytical performance of these POC showed high specificity, but sensitivity was lower in comparison with laboratory reference standards [30]. Our findings demonstrated that the RightBiotic provided AST results within 4 hours, as compared to the VITEK 2 system which typically required 26-30 hours. However, RightBiotic was low on criteria of sensitivity and specificity. In a previous study that evaluated the MBS POCT performance in estimating the bacterial load for UTI diagnosis, the presence of significant bacterial infections was detected in as little as 5 hours [31]. New methods such as T2MR POC can potentially provide even faster testing turnaround times [18].

Our analysis yielded discrepancies in the AST results between the two methods. Major errors in AST were observed for Gentamicin, Amikacin, and Imipenem and minor errors were observed for Gentamicin and Amikacin in one sample. These discrepancies result in false positive and negative results, suggesting the operational efficiencies of RightBiotic needs further optimization for accuracy and reliability. Similarly in a previous study from Arienzo et al. group, Flexicult POC was used in urine samples for AST and compared with standardized laboratory testing, Blom et al. reported a 4% error rate for assessing bacterial identification and quantification and correctly determined susceptibility in 93% of cases tested [32]. A different study comparing the use of Flexicult POC with conventional laboratory-based urine cultures found no changes in the use of antibiotics, recovery, patient empowerment, UTI recurrences, reconsultation, or hospitalisations [33]. On clinical grounds, RightBiotic diagnosed positive samples which were identified sterile through conventional methods, highlighting the need for further investigation. Shaw et al. also reported the similar problems with urine samples [34]. POC testing will undoubtedly have an impact on microbiology since it increases access to care and enables rapid detection of

infectious illnesses, which in turn allows for early and effective patient-centered therapy and management. It should be pointed out that, as with other diagnostic tests, the sensitivity and specificity will never be 100%, therefore some patients will need to be treated on a clinical basis. POCTs in routine clinical practice could potentially have an important impact, allowing bacterial identification and susceptibility testing in less time, by reducing analytical time and labor and diminishing analytical and management costs.The rapid response of the technology might limit the use of empirical therapy and prevent the misuse and overuse of antibiotics, which are primary factors driving the evolution of resistant bacteria.

Our findings confirm the utility of RightBiotic in compressing testing turnaround time of AST results from 26-30 hours required in conventional methods to 4 hours. This compression can potentially lead to improved patient outcomes by allowing for timely initiation of targeted antibiotic therapy. In a recent study, it was reported that patients with septic shock had a survival rate of 83% when treated within the first 30 minutes of symptom onset, whereas the survival rate decreased by 6% for every 30 minutes delay in treatment [35]. By shortening the time to receive susceptibility information from 2-3 days to just 4 hours, POCT technologies can facilitate more informed treatment decisions and contribute to the prevention of antibiotic misuse and overuse. However, to realize this potential, the technology must provide accurate and reliable results, which in our analysis was not provided by RightBiotic. This incorrectness heightens the risks of incorrect diagnosis and higher costs associated and loss of time with follow-up testing [36]

Our cost-effectiveness analysis, often not taken into account in POCT testing studies [37–39] showed RightBiotic to be less expensive as compared to conventional laboratory methods.The results of this analysis are consistent with other studies that also found POC testing to improve cost effective diagnosis [41,42]. It is essential that new health POC technologies are evaluated for their cost-effectiveness and their ability to help health systems maximise value for money and prevent waste of limited clinical resources in order to get the greatest possible health benefits from restricted clinical resources. RightBiotic was found favorable on cost-effectiveness criteria, encouraging its adoption by public health systems [42].

Conclusions

POCT can be important tools in the fight against AMR, particularly in community based settings of LMICs. If successful, POCTs can potentially help mitigate the risks arising out health inequities in terms of access and utilization of diagnostic services. However, we found that while the tested technology was founded favorable in terms of reducing testing turnaround time and cost-effectiveness (as compared to conventional testing methods), it did not reach the desired level for accuracy and sensitivity of results. This limitation far outweighs the potential benefits, as it can lead to incorrect diagnosis, treatment failures, and in cases even avoidable deaths. We acknowledge our study is based on a small statistically insignificant sample. However, the aim of the study was not to establish statistical significance, but to assess the practical feasibility of the technology to be adopted for clinical practice within a community setting. But we believe our study provides an objective assessment of the potential strengths and weaknesses of POCT technologies, which can help guide future research and development efforts, to realize their potential for clinical practice.

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